EFFECTS OF STRESS AND NICOTINE ON COGNITIVE FUNCTION IN MALE AND FEMALE RATS

by

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Thesis submitted to the Faculty of the
Medical and Clinical Psychology Graduate Program
Uniformed Services University of the Health Sciences
In partial fulfillment of the requirements for the degree of
Master of Science, 2016

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ACKNOWLEDGMENTS

I would like to express the deepest appreciation to my committee chair, Dr. Neil Grunberg who has helped me with this entire process and ensured that I put out the best product possible. I am grateful to USU for funding this project (Intramural Grant: E0721944). I would like to thank my committee members, Dr. Marian Tanofsky-Kraff and Dr. Andrew Waters for their dedication to my thesis. In addition, I would like to thank my labmates Aaron Weisbrod, Amanda Webb, Erin Barry, Maggie Baisley, Alice Graham, and Matthew Moosey for all their contributions to the data collection for this study. I would also like to thank the Laboratory of Animal Medicine, Graduate Education Office, the Uniformed Services University of the Health Sciences, and the Department of Medical and Clinical Psychology for providing me with the support needed to conduct this experiment.

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May 20, 2016

ABSTRACT

Title of Thesis: The Effects of the Stress and Nicotine on Cognitive Function in Male and Female Rats

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Cigarette smoking is the single most preventable cause of death and illness in the U.S., yet > 40 million Americans continue to smoke. The present experiment was designed to determine whether nicotine (addictive substance in cigarettes) alters cognitive effects of stress and to determine if females and males are affected differently. The present experiment used a well-established rat model to examine effects of nicotine or saline administration and a Warrior Stress Paradigm (WSP). The present experiment used 32 male and 32 female Sprague-Dawley rats. Both sexes were used because it is important to study the difference in responses. The dependent variables were acoustic startle reflex (ASR) without and with pre-pulse stimuli and pre-pulse inhibition (PPI) to measure startle responses, information processing, and sensory gating. The findings reveal several effects of stress and nicotine that differ in females and males. Males that received nicotine and stress had lower startle responses than males that received saline, $F(1,13)= 4.991$, $p=.044$, $\eta^2= .277$. There was a trend that when non-stressed males received nicotine, they had greater startle responses than non-stressed males that received saline, $F(1,13)=4.459$, $p=.055$, partial $\eta^2=.255$. Stressed females that received saline had sensory gating abilities, while non-stressed females did not, $F(1,27)=5.229$, $p=.030$, $\eta^2=.162$. If the present findings with rats extrapolate to the human condition, then nicotine may have more cognitive enhancing effects for women than men. If this

prediction is true, then adjusting smoking cessation strategies based on gender and life situations (e.g., amounts and types of stress) may be particularly valuable.

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CHAPTER 1: Introduction

TOBACCO & NICOTINE USE

Tobacco is one of the most frequently used substances in the world. As of 2013, 17.8% of adults aged 18 and older smoke cigarettes in the United States. While the prevalence of smoking is a decline from the 20.9% prevalence presented in 2005, the number of deaths attributed to smoking is a pandemic (82). Tobacco use is also more prevalent in the military population than the general population (6) (see Current Experiment). Tobacco causes more than 5 million deaths per year worldwide, and more than 480,000 deaths per year in the United States, which includes about 41,000 deaths as a result of secondhand smoke exposure (82). The amount of deaths caused by smoking is greater than the deaths of HIV, illegal drug use, alcohol use, motor vehicle injuries, and firearm-related incidents combined (67).

Smoking cigarettes has many detrimental effects to an individual's health. Cigarette use leads to an increased risk of developing cancer of the oral cavity, pancreas, and lung (81). There are two phases of cigarette smoke, tar phase, and gas phase. There are more than 10^{17} free radicals per gram in the tar phase of nicotine and more than 10^{15} free radicals per gram in the gas phase of nicotine. Free radicals are involved in chemical carcinogenesis and the concentration of free radicals in the lungs are increased with cigarette smoke (16). Smoking can cause detrimental effects to the majority of organs in the body. Smoking also affects dental health causing tooth loss (82), increases risk for cataracts causing impaired vision (82), and is also a cause for type 2 diabetes mellitus (70; 82). Within the United States, smoking causes 87% of lung cancer deaths, 32% of coronary heart disease deaths, and 79% of all chronic obstructive pulmonary disease

(COPD) cases (82). Despite the health consequences attributed with the use of tobacco, tobacco use is still quite prevalent. Given the multitude of negative consequences to tobacco use, it is understandable to question the reasons stated for continuing tobacco use. Many smokers report continued tobacco use to relieve stress (64); however, nicotine also plays a major role in the dependence upon tobacco (21; 44).

Nicotine is the component in tobacco products that causes addiction (21; 44). Nicotine acts on many facets of the body through absorption and can be absorbed in multiple ways including through the skin, mucous membranes, lungs, and gastrointestinal tract (39). Nicotine also affects the central nervous system (CNS), the peripheral nervous system (PNS), and cognitive function. The effects of nicotine on the body include feelings of stimulation or relaxation (39) and may have anti-depressive effects (68). Nicotine also raises the level of cortisol in humans and corticosterone in animals. Cortisol is a glucocorticoid that is released in response to stress. While people often list stress relief as a reason for smoking cigarettes, when combined with stress, nicotine has additive effects of increasing blood pressure, heart rate, and cortisol levels (52). The purpose of the present experiment is to investigate cognitive effects of nicotine and stress on female and male rats. Previous research has revealed a persistent difference in female and male stress reactions (11; 28; 71). Because of the difference in female and male stress reactions, it is necessary to study the cognitive effects of nicotine and stress in both sexes. The current experiment used an animal model for ethical considerations (see Current Experiment). This paper discusses tobacco use in the military, the effects of nicotine on cognition, combat stress, and the current experiment.

CURRENT EXPERIMENT

The current experiment investigated effects of nicotine and stress on cognition, including information processing, attention, and sensory gating in female and male rats. Nicotine was chosen to study because it is the addictive component in tobacco, which is a substance that has a high prevalence of use in the general population (82) and the military (7). While it is well known that smoking is detrimental because of the thousands of toxic chemicals in tobacco (16), nicotine, in contrast, has been reported to enhance cognition. Cognition is an important mental process and is involved in processes used every day including language, memory, and attention. Previous studies have indicated that nicotine is beneficial for reaction time and cognitive performance in individuals with pathological disease states such as Alzheimer's, Schizophrenia, and Attention Deficit Hyperactivity Disorder (34; 46; 56; 58; 69; 72).

The current experiment also used a military-relevant stressor on a male and female population. The military is increasing the availability of combat roles, and combat training opportunities for females (62; 85), and given the difference in male and female stress response (3; 8; 30; 96; 105), it is necessary to study the impact of military relevant stressors on females as well as male (see females in combat section). This experiment assists in contributing to the lack of research on females and their response to military relevant stressors. The military also has a high prevalence of tobacco use, with a prevalence rate of 24%, tobacco use is higher in the military than in the general population (20%) (7). The unique stressors experienced within the military population, especially during combat (79), also contributes to the need to conduct analysis of the effects of military relevant stressors, nicotine, and cognition in males and females. An

animal model was used in the current experiment because it would not be ethical to purposefully expose humans to stress, or expose them to an unnecessary surgical procedure for nicotine administration. The animals were treated ethically throughout the study.

TOBACCO USE IN THE MILITARY

Tobacco use, including the use of cigarettes and smokeless tobacco, is prevalent in the military. While less than 1 in 5 Americans used tobacco in 2011, 24% of active duty military personnel reported currently smoking (7). The prevalence of smoking varies by each military service. The military service with the highest reported rate of smoking is the U.S. Marine Corps (30.8%), followed by the Army (26.7%), the Navy (24.4%), and the U.S. Air Force (16.7%) (7). The use of smokeless tobacco is also much more prevalent in the military population when compared to the general population, such that, 49.2% of military personnel reported using a nicotine product, including cigarettes and smokeless tobacco, in the past 12 months (7). In 2011, 3.2% of the general population used smokeless tobacco, in comparison to 12.8% of military personnel who reported using smokeless tobacco in the past month. The prevalence of smokeless tobacco use also varies by each military service and follows the same pattern as smoking prevalence with the highest rate of smokeless tobacco use in the Marine Corps (21.3%), followed by the Army (13.7%), the Navy (10.7%), and the Air Force (8.7%) (7)The use of tobacco is particularly detrimental in a military context because it may negatively affects a soldiers' ability to maintain physical fitness and adequate endurance. The use of tobacco also compromises troop readiness and leads to significant health care costs. Military personnel who use tobacco often contribute to the debilitating sum of more than $1.6

billion each year that the DoD spends on tobacco-related medical care, increased hospitalization, and lost days of work (99). In addition to the health implications of the use of tobacco, there can also be implications on job performance. A previous study revealed that tobacco smoking (not nicotine per se) caused significant cognitive impairments including sustained attention, spatial working memory, strategy use, and executive planning in adults ages 18-29 (15). This finding is especially relevant given that 43% of active duty military are ages 25 and under (25). Military personnel can also experience cognitive decline during cessation attempts of tobacco. In 2003, Giannakoulas et al., investigated the effects of pilots who were required to abstain from smoking during flight. The pilots experienced nervousness, difficulty concentrating, and impairment of judgment (36). Military personnel are often tasked with assignments that can put not only their life in danger, but the lives of others in their command. Because of the immense responsibility that military personnel face, they must be alert at all times with proper cognitive functioning and tobacco can impede upon the cognitive functioning of individuals. The current experiment contributes to the literature regarding nicotine and the effects it has on cognition when combined with military relevant stressors.

SMOKING & COGNITION

Tobacco smoke contains 7,000 chemicals including chemicals, including heavy metals, free radicals, and nicotine. Many of these chemicals, such as hydrogen cyanide, arsenic, and vinyl chloride, are associated with brain toxicity and vinyl chloride is a risk factor for brain cancer (93). The heavy metals in tobacco smoke also are detrimental for cognition, as previous research has indicated that a lifetime exposure to lead is associated with lower levels of cognitive functioning, such as processing speed, verbal memory, and

learning (84). Previous studies have also suggested that current smoking status is predictive of cognitive impairment (14). Smoking is associated with an increased decline in cognitive factors, such as verbal memory and visual search speeds (77), and smoking can have a detrimental effect on reaction time and attention (27). Although smoking has been found to have a negative effect on cognition (15; 99), nicotine has been reported to have a positive effect on cognition (34; 56; 58). Therefore, it is valuable to research the possible favorable effects of nicotine and cognition with a military relevant stressor such as in the current experiment.

NICOTINE & COGNITION

Nicotine, the addictive substance in tobacco, is derived from the dried leaves and stems of the *Nicotiana Tabacum* and the *Nicotiana Rustica*. Nicotine can enter the body multiple ways including orally, through inhalation, and trans-dermally. Nicotine is a water and lipid soluble, liquid alkaloid that may be absorbed via respiratory tissue, skin, gastrointestinal tract, and mucous membranes. When tobacco smoke reaches the lungs, it is quickly absorbed due to the large surface area of the alveoli and small airways and the physiological pH of nicotine expedites transmission through cell membranes (45). The effects of nicotine are through nicotinic acetylcholine receptors. The stimulation of nicotinic acetylcholine receptors are responsible for the release of neurotransmitters and hormones (57) at autonomic ganglia, sensory nerve endings, neuromuscular junctions, and adrenal medulla (21; 44). Previous research has indicated the nicotine and nicotinic stimulation can be beneficial for cognition in humans and animal models (34; 56; 58). Nicotine improves reaction time in individuals, regardless of smoking status, and abstinence from smoking resulted in slower response times (46). A previous study

examined the effectiveness of nicotine on cognition by utilizing low nicotine and high nicotine cigarettes (72). The results of the study revealed that the high nicotine cigarettes improved immediate and delayed memory, while the low nicotine cigarettes were less effective in improving immediate and delayed memory. Nicotinic stimulation in humans is suggested to be beneficial in increasing the cognitive performance of individuals with pathological disease states, such as attention deficit/hyperactivity disorder, although it was not found to be beneficial in individuals not suffering from pathological disease states (69). In addition, nicotine also has been researched for its role in increasing sensory gating abilities (3; 20). Sensory gating is the ability to filter out unnecessary stimuli. It is imperative for accurate information processing and attention. An individual is unable to acknowledge information and manipulate it (information processing) or focus on a function of interest (attention) if he/she is overloaded with irrelevant stimuli. It is because of this that sensory gating it is a factor of interest for the current experiment. Although there are many detrimental effects for the use of tobacco use, there is a growing body of research that suggest nicotine itself can be beneficial in multiple ways, including cognition. Although nicotine itself may be beneficial for cognition, the general population does not have access to pure nicotine, but tobacco instead. While the negative effects of tobacco use are known, tobacco use is still quite prevalent in the general population (82), and the military (7). Within the current experiment the effects of nicotine will be investigated regarding its effects on cognition and will be administered via osmotic mini-pump because it has been successfully used as a nicotine administration method in previous studies (3; 39; 43; 68; 105).

STRESS

One definition of stress is the body's response to a threat (91). This threat may be physical or psychological and can result in different bodily responses (91). A behavioral response is how an individual's body reacts to the occurrence of stress (54). Experiencing stress is an unavoidable part of life, but excess amounts of stress can have detrimental effects on the physical and mental health of an individual (54). Individuals who are members of the military may be exposed to a greater amount of stress than civilians (79), and this stress increases following exposure to combat (86).

Stress can be categorized according to numerous variables such as the type of stress, the duration it is experienced, and the type of response to the stressor (54). Eustress is a positive stress that motivates you to complete actions. Distress is a negative stress that can cause detrimental effects on the body. Stress also can last and occur for different amounts of time. Acute stress is the most frequently experienced type of stress. It occurs for short periods of time and often as a result of daily activities such as trying to meet a deadline. Chronic stress occurs as a result of prolonged stressors such as being in a tasking job position. Chronic and acute stress also have different effects on the body. Acute stressors have been attributed with causing significant changes in the central nervous system (CNS), whereas chronic stress has been attributed with changes in the immune system (89). The responses to stress can be differentiated into various categories such as physiological, affective, behavioral, and cognitive (54). Because of the prevalence of tobacco use in the military, it is imperative that the effects on behaviors such as information processing, attention, and sensory gating are understood to ensure

that the use of tobacco does not hinder the ability to adequately complete a task, thereby putting individuals in harm's way. It was the aim of this study to investigate the effects of stress and nicotine on the cognitive functioning of females and males through the use of an animal model.

COMBAT STRESS

There are certain factors that may put individuals at a greater risk of experiencing stress, including one's occupation (51). One occupation that is described as having a large amount of stress is being a member of the military (79). A 2002 study conducted on work stress in the military found that individuals in the military were significantly more likely to report suffering from stress than civilian workers (73). While being in the military exposes those individuals to increased stress levels, deployment and exposure to combat yields another level of stress. Stressors that are experienced during deployment and combat include physical stressors, such as exposures to extreme heat, cold, dehydration and wetness (32) as well as cognitive stressors, such as uncertainty due to soldiers not receiving enough information about a mission (32).

Deployment and exposure to combat also increase the possibility of suffering from PTSD and PTSD symptoms. Smith et al. (86) conducted a study to investigate the onset and persistence of PTSD after deployment and combat related exposures. This study reported a threefold increase in the new onset of self-reported PTSD symptoms or diagnosis among the military personnel who reported combat exposures (86). Combat exposures can include the exposure to an enemy soldier (predator). Predator stress occurs from an individual experiencing a significant threat of injury or death (91), and the Warrior Stress Paradigm (WSP) (see methods section for detailed description) seeks to

model this type of stressor. The chronic stress endured during deployment can be detrimental to soldiers both mentally (e.g., anxiety, depression) and physically (e.g., chronic fatigue syndrome) (79). The current experiment the WSP, which involves a chronic predator stressor along with unpredictable non-painful environmental stimuli.

FEMALES IN COMBAT

There are approximately 203,000 women in the United States military (85). As of 2009, women comprised 14.5% of the total active force of the U.S. military (85). Presently, women comprise 20% of new recruits for the military (6), and it is estimated that the female veteran population will increase from approximately 10 to 18% by the year 2040 (6). The "risk rule," that was enacted in 1988 (106), which limited a women's ability to be attached to combat units (92), was recently rescinded. With the restriction to obtain combat positions removed, women in the military will have greater exposures to combat and therefore combat-related stress. It is also important to acknowledge that with the evolution of women and their increasing combat roles, there is also a transition to expose women to more severe stress in training. Across the military, schools are opening up such as ranger school, and navy seal school where women are being put in incredibly stressful situations before being exposed to combat (62). Previous research has indicated that exposure to combat yields another facet of stressors (32; 86), and likelihood of developing PTSD (86). The current study included females to further investigate the effects of combat related stressors, because females will be more prevalent in combat units.

SEX DIFFERENCES IN THE STRESS RESPONSE

Females and males have a different response to stress. Walter Cannon established the "fight or flight" stress response which describes the human response to stress or danger (48). The "fight or flight" response states that when presented with a threat, the body will prepare to fight or flee (48). A biological basis providing further support for this stress response was analyzed through a study that discovered the activation of the sympathetic nervous system as a result of an imposed threat (49). Although, these studies were based on data collected from males, the results were generalized to the stress response for females and males.

Taylor, Klein, and colleagues (96) formulated an alternative response to stress in females, called "tend and befriend." This alternative response to stress filled an empirical gap within stress research because during that time there was a gender bias with the majority of research being conducted on male populations. The theoretical model of "tend and befriend" indicated that there is biobehavioral support for the "tend and befriend" response to stress in females which was the attachment/caregiving system. This system was stress-related and although it has previously been researched for its role in maternal bonding and child development, Taylor, Klein, and colleagues suggested that it also has implications for the stress response of females. The "tending" behavior involves activities that protect the self and offspring, while the "befriending" behavior involves social enrichment that provides the group with a greater ability to detect predator, and chances of a predator attacking a group is less than the chance of a predator attacking an individual (96). Further investigation into this stress response discovered that the oxytocin release in females and males differs when there is an encounter of stress, which may account for the difference in stress responses across genders (95; 96).

Previous animal studies have been conducted that further support the hypothesis that females and males react differently to stress. Studies have discovered a difference in the responses of females and males in response to social stress (11), restraint stress (28), and predator stress (71). Social stress was investigated by manipulating the housing conditions of male and female rats by placing the rats in crowded housing versus individual housing. The female rats that were placed in individual housing had higher levels of corticosterone (biological measure of stress in animals), while the male rats had higher levels of corticosterone when placed in crowded housing (11). Faraday (28) investigated the differences of rat sex differences in response to stress and found that restraint stress significantly decreased the feeding and body weight of male rats, but did not significantly decrease the feeding and body weight of female rats. Decreases in food intake and/or body weight were used as a sign of stress in animals (11). Park et al. (71) also investigated sex differences and the effects of acute predator stress on spatial learning and memory. The results stated that while male and female rats both expressed impaired short-term memory following exposure to predator stress, females exhibited greater baseline and stress-evoked responses than males (71). It is evidenced in previous studies that chronic stress has significantly different effects on females and males even when different types of stressors are considered. The evidence also reveals the necessity of using a statistical analysis method that will take the baseline differences between females and males into account (11; 28; 71). Unfortunately, even with the wealth of studies conducted on animal models, and responses to stress, the majority of published studies were conducted only on males. With the apparent difference in the response of

stress between females and males, it is necessary to conduct more research studies on the effects of stress on female animal models in comparison to male animal models.

ANIMAL MODELS OF STRESS

The current experiment utilized a Warrior Stress Paradigm (WSP). The WSP models chronic predator and environmental stress, a type of repeated psychological stress, on male and female Sprague Dawley rats and the cognitive effects it has on the animals were measured (8; 104; 105). The WSP was designed to model combat stress experienced by military personnel. Military personnel has a high prevalence of tobacco use (7), and are therefore a population of interest for the effects of nicotine (the addictive substance in tobacco), cognition, and a military relevant stressor. As of September 2011, 43% of active duty members of the military are ages 25 or younger (25). Therefore, the age of the rats used in the study were early adulthood to model the prevalence of this age group in active duty military members (29; 90). Predator stress has been modeled in many types of studies with both the use of a live animal (33; 71) and the use of animal scents (18; 19; 33; 71). The animal models of predator stress are especially useful because they do not utilize a method that could cause the animal physical pain such as the electric shock method (33). The use of animal models in order to study specific psychiatric behaviors is prevalent in research. However, it is understood that use of animals is to model human behaviors and in no way make the assumption to translate perfectly to humans.

The behavioral measure that was used in the current study are acoustic startle response (ASR) with and without pre-pulse (see methods section). The research study involved a surgical placement of a mini-pump for nicotine administration and exposure to a repeated stressor. It is unethical to purposefully expose humans to stressors and unnecessary surgery, therefore an animal model was the most appropriate method for investigating the effects of nicotine and the WSP on the cognitive function of females and males. The use of an animal model also grants the researcher a greater amount of experimental control. The inclusion of male and female rats allows for determination of any sex differences, because previous experiments have revealed differential effects of nicotine in male and female rats and humans. In addition, NIH regulations for animal research require the inclusion of males and females, unless there is a particular reason to exclude on sex or the other, in all animal research (17).

CHAPTER 2: Overview and Specific Aims

The present experiment was designed to determine effects of nicotine and psychological stress on cognitive function of male and female rats. There were three specific aims: (1) to determine effects of a military-relevant stressor (threat of attack) on behaviors (cognition) in male and female rats; (2) to determine whether nicotine alters the effects of stress; and (3) to determine if females and males are affected differently by the combination of stress and nicotine. The cognitive functioning of the rats was measured by Acoustic Startle Response (ASR) with and without Pre-pulse inhibition (PPI) at baseline and two subsequent time points (See Figure 23). This experimental design was conducted utilizing an animal model of male and female Sprague Dawley rats.

Specific Aims/ Hypotheses

Specific Aim 1: To determine effects of a military-relevant stressor including threat of an attack by a predator (fox urine) and non-painful unpredictable environmental stimuli in male and female rats.

Hypothesis 1: Stress will deleteriously affect cognition (impaired attention and information processing) that will be detected by a decrease percent pre-pulse inhibition in ASR.

Rationale. Many studies that have analyzed effects of stress on cognition. Previous studies have reported that stress has a negative effect on cognition, including learning and memory (65), declarative memory (66), and cognitive function (61). Stress also has been shown to affect the structure of certain parts of the brain including the hippocampus, which is associated with learning and memory (9) and verbal declarative memory (10), and the amygdala, which plays an integral role in fear response. Traumatic

stress has been reported to alter neural circuitry of the prefrontal cortex, which modulates the emotional responsiveness through inhibition of amygdala function (10).

Specific Aim 2: To examine the cognitive effects of nicotine.

Hypothesis 2: Nicotine will attenuate deleterious effects of stress on cognitive measures (i.e., attenuate deleterious effects of stress on attention and information processing).

Rationale. Given the previous research on the robust positive effects of nicotine on cognition (34; 56; 58), it is hypothesized that the positive effects of nicotine will attenuate the detrimental effects of stress. Nicotine activates nicotinic receptors. Nicotinic receptors have previously been found to be integral in the maintenance of ideal performance on cognitive tasks (56). Nicotinic agonist treatment has also been successful in improving attention, learning, and memory (60). Nicotine agonist treatment improves attentional performance in Alzheimer's disease, schizophrenia, and attention deficit hyperactivity disorder (58).

Specific Aim 3: To determine if females and males are affected differently by the combination of stress and nicotine.

Hypothesis 3: Females will be particularly sensitive to effects of stress and to effects of nicotine on stress (i.e., nicotine will be more beneficial for female than for male rats under stress).

Rationale. Nicotine has been reported to have greater protective effects in females than in males, including anti-depressive effects (68) and stress-induced mood changes (34).

CHAPTER 3: Methods

This experiment was a 2 (saline, 6 mg/kg) x 2 (no stress, Warrior Stress Paradigm (WSP) x 2 (male, female) full factorial mixed design conducted with rats as subjects. The experimental design has been widely used in animal experiments examining behavioral and biological effects of stress and nicotine (3; 4; 30; 42). This experimental design resulted in eight experimental conditions. There were eight subjects in each treatment condition (Table 1). The number of subjects per condition was based on previous research by the Grunberg Laboratory with similar paradigms that yielded significant results (3; 30; 68). This experiment utilized a total of 64 subjects. The total subjects were divided into two separate counter-balanced cohorts of 32 subjects. The independent variables were nicotine (saline and 6 mg/kg), stress (no stress and WSP), and sex (male and female). The dependent variable was the behavioral measures acoustic startle response with and without pre-pulse. ASR provides information about information processing, attention, and sensory gating abilities. Animal husbandry conditions, independent variables, dependent variables, experimental timeline, and data analytic strategy are explained in greater detail below.

ANIMALS AND HOUSING

This study consisted of 64 Sprague Dawley rats received from Charles River Laboratories, 32 male and 32 female. Sprague Dawley rats were the strain chosen because of their prevalence in animal models of stress studies (19; 26; 71; 104). The rats were 54 days old upon arrival. It has been determined by previous investigators that the adolescent period for female rats ends at 42 days, and 55 days for male rats (90). Adulthood begins around 60 days for the female and male rats (29). Therefore, the rats

used in this study were 54 days upon arrival to model late adolescence to early adulthood of military personnel. It is noteworthy that the animals studied in the present experiment also were used in another experiment in the Grunberg Laboratory (101). Therefore, some of the descriptions of methods are identical.

The rats were individually housed in standard polycarbonate shoebox cages (42.5 x 20.5 x 20 cm) with filter tops, and hardwood chip bedding (Pine-Dri). Individual housing was chosen because previous investigators have reported that social enrichment can affect behavioral and biological effects of the rats (26; 75). The cages were changed twice a week by the Laboratory Animal Medicine (LAM) husbandry staff to ensure the rats are residing in ethical and humane living conditions. The rats also had continuous access to food (Harlan Teklad 4% Mouse/Rat Diet 7001) and fresh water. The room that the rats were housed in maintained a temperature of 23°C with 40% relative humidity. The housing room also maintained a 12-hour reverse light cycle with lights out from 0500-1700. Rats are nocturnal animals and the reverse light cycle allows behavioral measures to be conducted during the rats' active phase (2; 68; 75; 104). The rats were numbered by markings that were placed on their tails with permanent marker. The rats also experienced a "gentling" period, during which they were handled for 5 minutes a day for their first two days at the facility. During gentling, rats were held, pet, and spoken to in soft tones so that they can become accustomed human handling and voices (40; 100). The gentling period is particularly important with this subject population, as Sprague Dawley rats are bred for research and do not come into contact with humans often. Previous research has shown that gentling rats has a long term effect of decreasing the

rats' fear of humans, which can be an additional stressor and confound the results of the study (63).

All experimental procedures and protocols were approved by the Uniformed Services University of the Health Sciences (USUHS) Institutional Animal Care and Use Committee (IACUC; protocol: MPS-14-898) (see Appendix F). The procedures were conducted according to the NIH Guide for Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1985). This experiment was conducted with every effort to minimize the number of rats that were used and to observe and minimize their discomfort during participation.

INDEPENDENT VARIABLES

The independent variables of the study were nicotine, stress, and sex. Each of the independent variables had two levels and is discussed in further detail below.

Nicotine

Nicotine is the addictive component in tobacco (USDHHS, 1988) - one of the most widely used drugs in the world. While there are hundreds of chemicals in cigarettes, nicotine has previously been studied for the possibility of being beneficial in human and animal studies and has previously reported improvement of cognitive and motor performance (1; 3; 74; 78) and depression in an animal model (68). The prevalence of cigarette use in the general population calls for research on both the negative and positive effects of nicotine, especially in conjunction with stress, because the majority of individuals report the anxiolytic effects of smoking as the reason for continued tobacco use (50). Nicotine bitartrate (Sigma Pharmaceuticals) was chosen as the chemical for the independent variable because it has been previously used in

successful animal and human studies (3; 68; 104; 105). The subjects were placed in a saline group or nicotine group. The nicotine group received 6 mg/kg nicotine bitartrate dissolved in saline. The nicotine bitartrate was expressed as a nicotine base. The dosage of nicotine was chosen because it has previously yielded results in rats analogous to the effects of humans smoking ½ pack to 1 pack of cigarettes per day (103). This dosage also has yielded nicotine and cotinine levels comparable to humans who smoke tobacco (102). The nicotine and saline dosages were administered via osmotic mini-pump (Alzet Model 2002, Durect Corporation). The amount of nicotine bitartrate solution in each mini-pump was calculated according to the average weight of the rats in each group.

The osmotic mini-pump was surgically implanted subcutaneously between the withers of the rat. Surgery staff recorded the time the animals went under anesthesia, surgery start and stop time, time the animal was returned to its cage, and time the animal was alert and moving around. These times were observed and recorded to make sure that there were no outliers and to take note of any rats that may need further observation. The rats were under anesthesia (5% isoflurane/oxygen mixture) during the surgical procedure. The rats were injected, in the Gluteus Maximus, with buprenorphine (buprenex) to serve as an analgesic before the surgical procedure was initiated. The fur between the withers of the rats was shaved and betadine was placed on the shaved site to prevent contamination during surgery. Blunt nosed scissors were used to cut the flesh of the rats, making a 1cm incision, and create a pocket to insert the mini-pump. The implantation site was then closed with 9mm wound clips. The rats were placed in clean cages and were observed until they awoke from anesthesia and were then returned to their housing room.

The weights of the rats were recorded for three consecutive days before the surgery. The weights were recorded to ensure that the subjects were not showing any physical signs of distress including weight loss of greater than 10%. The recorded weights were used to calculate the dosages of nicotine bitartrate and saline solution for the osmotic mini-pumps. Male and female rats differ considerably in their weights, and the amount of bitartrate dissolved in saline was calculated to deliver 6 mg/kg. Therefore, it was necessary to conduct separate nicotine bitartrate calculations for the males and the females (38; 40; 42). The three recorded weights of the rats were averaged and used for the nicotine calculations. The rats were divided into four groups (male saline, male nicotine, female saline, and female nicotine) according to their weights. It was important to make certain that the weights of each group for females and males were comparable between groups to decrease the possibility of confounding variables. Following the division of the rats into saline and nicotine groups the range, minimum, maximum, mean, and standard deviation of each group was calculated. This division was completed as a safeguard to ensure that the groups were comparable in descriptive statistics. SPSS 22 was used for these calculations.

Following the body weight calculations and the division of the rats into saline and nicotine groups, the nicotine calculations were completed. The mini-pumps chosen for the surgery came from the same manufactured lot. Each lot has a certain *in vitro* pump rate, duration, and mean pump fill volume. Therefore, it is important to remain consistent with the lot used for each rat. The nicotine calculations ensure that an accurate nicotine dosage is being placed in each mini-pump and that the pump duration and fill volume will last throughout the entire experiment. The mini-pump surgery was conducted in the

Laboratory of Animal Medicine (LAM) at USUHS. The rats were operated on in a surgical environment while under anesthesia (5% isoflurane/oxygen mixture), injected with buprenorphine (buprenex) in the Gluteus Maximus, before being placed under anesthesia for pain reduction, and placed in clean cages following implantation of the mini-pump. The rats were observed while awaking from anesthesia to ensure that there were no adverse effects, and the incision was checked daily for signs of infection.

Warrior Stress Paradigm (WSP)

Military personnel are often exposed to adverse and unpredictable situations during deployment. Soldiers are frequently faced with life threatening situations while residing in an unfamiliar and hostile environment. Military personnel who are exposed to combat often experience stressors, including difficult living and working environments, perceived threat, as well as nuclear, biological, and chemical exposures (97). The WSP for rats was created to model the stress commonly experienced by military personnel during deployment, including unpredictable environmental stimuli and exposure to a predator (predator stress) (104; 105). The use of the WSP allowed for a true experiment with careful manipulation of stress and the environment the paradigm was conducted in.

Predator Stress.

There have been previous studies conducted to model the predator stress paradigm utilizing a live animal (33; 71) and the scent of a known predator (18; 19). The predator stress paradigm is an ethologically relevant model of stress in the rodent species because it produces activation of the Hypothalamic-Pituitary-Adrenal (HPA) axis and the central release of stress relevant neurotransmitters and messengers (33). Activation of the HPA axis also activates the immune system and relevant cytokines involving immune

function and response (19). The use of the predator stress paradigm allows for investigation of the effects of stress on multiple variables. These paradigms have been utilized for animal models investigating its effect on the behavioral responses to stress such as anxiety-related and depressive-related behaviors and biomarkers of the immune system, such as cytokines (18; 19; 33; 71). The predator stress manipulation allows exposure of sensory stimuli without the presence of a live predator (8; 71). There have also been previous studies conducted on animal models that utilize a predator stress paradigm (43; 68; 71; 104), however none of these studies have looked specifically at the effects of nicotine and WSP on the cognitive functioning of the subjects.

Stress Manipulation.

The WSP begins with the rats being transferred to a neutral lab room with white lights where they are transferred from their original cages (42.5 x 20.5 x 20 cm) to individual cages (29 x 18 x 12 cm) without bedding. The transfer of the rats from the housing room begins the stressor process (104; 105). Sprague Dawley rats are nocturnal animals and their housing cages use hardwood chip bedding. The transfer to a smaller, mouse cage without bedding in a room with white lights acts as a stressor to the rats. The rats are exposed to a cotton ball with 10 mL of commercially purchased synthetic fox urine (Buck Stop, Stanton, MI) for 20 minutes on the first day of exposure. For days 2-14 the animals are exposed for 10 minutes/day to fox urine followed by 10 minutes/day of an unpredictable non-painful environmental stressor. Foxes are a natural predator of rats, therefore the scent of fox urine was chosen as an exposure to a predator scent during the WSP. The non-painful environmental stressors included noise, flashing lights, and cage shaking to avoid habituation to the fox urine over the 14 days of stress (Figure 21).

The group of rats that were not in the WSP group stayed in the housing room during the stress manipulation.

Sex

With the removal of the "risk rule," military women will be exposed to a multitude of new stressors that were not encountered in the past due to their job positions. While there has been a great deal of research conducted on combat-related stressors and their effects on the military (83; 87; 88), the majority of these studies have been conducted on males. This experiment was conducted with female and male subjects to examine and compare the effects of the WSP on both sexes. The results of this study provided information regarding the similarities and differences of the effects of nicotine and/or stress has on the cognition of females and males. There also have been previous studies conducted on animal models that utilize a predator stress paradigm (68; 71); however, none of these studies have examined effects of nicotine and WSP on the cognitive functioning of the subjects.

DEPENDENT VARIABLE

The dependent variable of the study was Acoustic Startle Response (ASR) with and without pre-pulse and pre-pulse inhibition (PPI). Each of the dependent variables are discussed in further detail below.

Acoustic Startle Response (ASR) with and without pre-pulse

ASR with and without pre-pulse provides a behavioral measurement of central information processing and attention. This behavioral measurement uses a startle reflex that is produced by an acoustic stimuli. A startle reflex is a fast motor response to a

sudden, intense stimuli. An example of a startle can include a twitch of facial or body muscles. An example of a sudden intense stimuli that can produce a startle include a tactile, visual, or acoustic stimuli. Startle reflexes are experienced by humans and animals. The startle response pattern in a rat consists of observable physical and physiological changes. Physical changes include an eye-lid closure, contraction of facial muscles, and lack of movement or additional movement. A physiological change can involve the acceleration of the heart. The startle response pattern is thought to be a protective response that prepares the subject from a predator and starts preparation for a fight-or-flight response. This hypothesis suggests that ASR will be enhanced in threatening situations or following an aversive event such as the WSP. Previous research has confirmed that ASR in rats is enhanced following an aversive event such as a fear potentiated startle (24), a loud noise (1; 3; 22; 35; 80), bright illumination (98), and electric shock (23).

The ASR behavioral test is an efficient behavioral measure because under the appropriate experimental conditions, the startle has a non-zero baseline and the effects can be enhanced and attenuated. The ability to enhance or attenuate the effects of the startle allows ASR to be a valuable tool to measure and assess mechanisms of sensorimotor response plasticity. ASR of humans and animals becomes functional immediately after the onset of hearing. The versatility of the ASR allows the measure to be used by a wide range of ages in humans and animals.

Pre-pulse Inhibition (PPI).

PPI occurs when a preceding weaker stimulus inhibits a strong ASR. PPI can cause a reduction in the ASR when it is presented 30-500 msec before the startling

stimulus (53). A pre-pulse stimuli can be presented visually or via auditory methods. PPI has previously been used as an operational measure for sensorimotor gating mechanisms (47). Sensorimotor gating is the ability to filter out unnecessary stimuli in the brain from environmental stimuli. An example of adequate sensorimotor gating is the ability to focus on the conversation with a person of interest at a cocktail party filled with other individuals. PPI is a well-established measurement of sensorimotor gating abilities (12). Previous research has indicated that PPI of ASR is reduced in certain mental disorders including schizophrenia, obsessive-compulsive disorder, and attention-deficit disorder (94). While these disorders may seem vastly different, each of the disorders is categorized by the inability to gate invasive sensory, motor, or cognitive information (53).

Data Recording.

ASR and PPI are measured in an Acoustic Response Test System (Med Associates). The test system includes weight-sensitive platforms and individual sound-attenuated chambers. The subjects are placed in the cage, which sits atop the weight sensitive platform, and their movements in response to the stimuli are measured as a voltage change by a strain gauge inside each platform. The test system is interfaced with a Nexlink computer to record the responses from the test system. The subjects are each placed in the individual sound-attenuated chamber in an experimentation room. Testing is conducted in red light so the animals do not encounter any additional stress. Startle stimuli are 110 or 120 dB. The startle stimuli are white noise bursts of 20 msec duration sometimes preceded 100 msec by 68 or 82 dB, 1kHz pure tones (pre-pulses). These

parameters are widely used in the literature. Each stimulus combination is presented 8 times. Total testing period is about 20 min.

ASR with and without pre-pulse.

Animals were allowed to acclimate to the chambers during two, 20 minute sessions (separate, but consecutive days) prior to other measurements. The baseline measurements of ASR with and without pre-pulse were collected before the nicotine mini-pump surgery (Day 8) and the initiation of the WSP (Day 10). There were two subsequent measurements of ASR. These measurements were designated as T1 and T2. The T1 and T2 measurements were taken after the nicotine mini-pump surgery. T1 was taken on day 19 and T2 measurement was taken on day 29.

EXPERIMENTAL TIMELINE

After the rats arrive at the facility, they were individually housed in their standard polycarbonate shoebox cages (42.5 x 20.5 x 20 cm) and placed in the housing room (70). The rats were numbered and encountered a "gentling" period, which occurred for 30 minutes a day for two days (Days 1 & 2). ASR acclimation consisted of two days for the rats and began on day 2 of the experiment. Baseline scores for ASR were recorded the next day (day 8). Surgery occurred on day 9 of the experiment and the WSP lasted from day 10 to day 16. There was a 3-day break in between stress days, for behavioral measurements, and then the WSP continued on day 20 to day 26. The behavioral measurements (ASR) were collected again on day 19 (ASR T1), and day 29 (ASR T2). Following the completion of the behavioral measures the rats were euthanized (day 30) and their trunk blood was stored for later analyses. Behavioral tests were not conducted on the same day. Figure 22 presents the experimental timeline.

DATA ANALYTIC STRATEGY

Repeated-measures analysis of covariance (rANCOVA) was used to analyze aims 1, 2, and 3. The acoustic startle response was analyzed with a rANCOVA for all levels of acoustic startle with and without pre-pulse (110 dB without pre-pulse [PP], 110 dB with 68 dB pre-pulse, 110 dB with 82 dB pre-pulse, 120 dB without pre-pulse, 120 dB with 68 dB pre-pulse, and 120 dB with 82 dB pre-pulse). Pre-pulse inhibition was calculated using the following formula: (amplitude without PP - amplitude with PP)/amplitude without PP x 100). The percentage was calculated for each ASR with pre-pulse (110 dB with 68 dB pre-pulse, 110 dB with 82 dB pre-pulse, 120 dB with 68 dB pre-pulse, and 120 dB with 82 dB pre-pulse). An rANCOVA was then conducted on each level of PPI. The data analysis was conducted using SPSS 22. These subscale scores were each analyzed using rANCOVA. The baseline score was the covariate for the activity and latency scores at T1 and T2. The rANCOVA for ASR, and PPI was also split for nicotine (0 mg/kg, 6 mg/kg), stress (no WSP, WSP), and sex (male, female), to evaluate the presented hypotheses. Analyses of ASR, and PPI included data for all subjects (N=64). All tests were two tailed using alpha = .05. Adjusted values were reported in the document due to baseline differences between females and males. Several steps were taken to reduce type 1 and type 2 errors. For example, the sample size that chosen was based on previous research experiments that yielded significant results and increased the power (3; 41; 68; 104; 105). The alpha level was .05 to prevent incorrectly rejecting the null when the null hypothesis is true.

ETHICAL CONSIDERATIONS

The animals in this study were treated ethically during their entire participation. The researchers upheld the humane treatment and care of the research animals according to the guidelines provided by the Institutional Animal Care and Use Committee (13). The animals were provided food and water and their cages were changed twice a week. At the end of the experiment, animals were euthanized using methods that minimize their suffering and distress. If there was an instance where a rat was having increasingly adverse reactions to participation in the study, then the rat would be euthanized to eliminate their pain. There was no instance, within the current experiment, where a rat had adverse reaction and had to be prematurely euthanized.

CHAPTER 4: Results

ACOUSTIC STARTLE RESPONSE (ASR)

A univariate analysis revealed significant differences among groups at baseline. Therefore, ANCOVAs were conducted using the baseline ASR values as the covariates. An overall ANCOVA for ASR, using all independent variables, was conducted to determine main effects of and interactions between variables. Following the revelation of a significant interaction, a univariate ANCOVA was conducted at each time point. The data were split by sex to explore analyses for females and males separately, and next split by sex and stress to explore analyses for nicotine effects within the different treatment groups.

Overall rANCOVA 110 dB with no pre-pulse. See Figures 1 & 2 and Table 2. There was a main effect of sex, $F(1,55)= 19.190$, $p<.001$, $\eta^2= .259$, such that females (mean [M]= 8.995, standard error [SE]= .521) had lower startle responses than males (M= 12.226, SE= .521). There also was a significant time x stress x nicotine interaction, $F(1,55)= 5.028$, $p= .029$, $\eta^2= .084$.

rANCOVA 110 dB with no pre-pulse, split by sex. See Figure 2, and Table 9. There was a significant interaction of stress x nicotine for males at T1, $F(1,27)= 4.212$, $p=.050$, $\eta^2= .135$, observed power=.508 such that males that were exposed to stress and saline had an increase in startle, whereas males that were exposed to stress and nicotine had a decrease in startle. There were no main effects or significant interactions for females at T1. There were no main effects or significant interactions found at T2.

rANCOVA 110 dB with no pre-pulse, split by stress and sex. See Tables 20, 21, 22, & 23. There were no main effects or significant interactions.

Summary. There was an overall significant time x stress x nicotine interaction and a main effect of sex, which revealed that females had lower startle responses than males. The data split by sex revealed a significant interaction of stress x nicotine for males revealing that the combination of stress and nicotine resulted in a decrease in startle response. The data split by sex and stress did not reveal any main effects or significant interactions.

Overall rANCOVA 110 dB with 68 dB pre-pulse. See Figures 3 & 4 and Table 3. There was a main effect of sex, $F(1,55)= 9.471$, $p= .003$, $\eta^2= .147$, observed power =.856, such that females (mean= 9.638; standard error= .526) had lower startle responses than males (mean= 11.931; standard error= .526).

rANCOVA 110 dB with 68 dB pre-pulse, split by sex. See Figures 1& 2 and Tables 10 & 11. There were no main effects or significant interactions.

rANCOVA 110 with 68 dB, split by sex and stress. There were no main effects or significant interactions.

Summary. There was an overall main effect of sex, which revealed that females had lower startle responses than males. The data split by sex did not reveal any main effects or significant interactions. The data split by sex and stress did not reveal any main effects or significant interactions. Therefore, only the sex difference was significant.

Overall rANCOVA 110 dB with 82 dB pre-pulse. See Figures 5 & 6 and Table 4. There was a main effect of sex, $F(1,55)= 9.848$, $p=.003$, $\eta^2= .152$, observed power =.869, such that females (mean= 9.067; standard error =.574) had lower startle responses than males (mean =11.631 standard error =.574).

rANCOVA 110 dB with 82 dB pre-pulse, split by sex. See Figures 5 & 6 and Table 4. There were no main effects or significant interactions.

rANCOVA 110 dB with 82 dB pre-pulse, split by sex, and stress. See Figure 6. There was a main effect of nicotine at T1 for males that received the WSP, $F(1,13)= 4.991$, $p=.044$, $\eta^2= .277$, observed power=.543, such that males that received nicotine (mean=10.399, standard error=.858) had a lower startle response than males that received saline (mean=13.141, standard error=.858). There were no main effects or significant interactions at T2.

Summary. There was an overall main effect of sex, which revealed that females had lower startle responses than males. Males that were exposed to the WSP and received nicotine had a lower startle response at T1 than males that were exposed to the WSP and received saline.

Overall rANCOVA 120 dB with no pre-pulse. See Figures 7 & 8 and Table 5. There was a main effect of time, $F(1,55)=6.101$, $p=.017$, $\eta^2=.100$, observed power=.680, such that T1 startle responses (mean=10.766, standard error=.395) were lower than T2 startle responses (mean=11.610, standard error=.470). There was a main effect of sex, $F(1,55)= 11.410$, $p= .001$, $\eta^2= .172$, observed power= .913, such that females (mean= 9.947, standard error= .503) had lower startle responses than males (mean= 12.430, standard error= .503). There was a significant time x stress x sex interaction, $F(1,55)= 4.882$, $p= .031$, $\eta^2= .082$, observed power= .584. There was a significant stress x nicotine interaction, $F(1,55)= 4.960$, $p= .030$, $\eta^2= .083$, observed power= .590.

rANCOVA 120 dB with no pre-pulse, split by sex. See Figure 8 and Table 5. There was a significant nicotine x stress interaction for males at T1, $F(1,27)=4.717$,

p=.039, η^2=.149, observed power=.553. There were no main effects or significant interactions at T2.

rANCOVA 120 dB with no pre-pulse, split by sex, and stress. See Figure 8 and Table 5. There was an effect of nicotine approaching significance at T1 for males that did not receive WSP, F(1,13)=4.459, p=.055, η^2=.255, observed power=.498, such that males that received nicotine (mean=13.876, standard error=1.234) had a greater startle response than males that received saline (mean=10.102, standard error=1.234). There were no main effects or significant interactions at T2.

Summary. There was an overall main effect of time and sex which revealed that startle responses at T1 were less than startle responses at T2 and that females had lower startle responses than males respectively. There was an overall significant time x stress x sex interaction and there was a significant nicotine x stress interaction at T1 for males.

Overall rANCOVA 120 dB with 68 dB pre-pulse. See Figures 9 & 10 and Table 6. There was a main effect of sex, F(1,55)= 29.717, p= .000, η^2= .351, observed power= 1.000, such that females (mean=9.252 standard error=.467) had a lower startle response than males (mean=12.864 standard error=.467). There was a significant time x stress x nicotine interaction, F(1,55)= 4.752, p= .034, η^2= .080, observed power= .572.

rANCOVA 120 dB with 68 dB pre-pulse, split by sex. See Figures 9 & 10 and Table 6. There were no main effects or significant interactions.

rANCOVA 120 dB with 68 dB pre-pulse, split by sex, and stress. There were no main effects or significant interactions.

Summary. There was an overall main effect of sex, such that females had lower startle responses than males. There was a significant time x nicotine interaction.

Overall rANCOVA 120 dB with 82 dB pre-pulse. See Figures 11 & 12 and Table 7. There was a main effect of time, $F(1,55)=7.25$, $p=.009$, $\eta^2=.116$, observed power=.753, such that T1 startle responses (mean=10.424, standard error=.352) was less than T2 startle responses (mean=11.701, standard error=.434). There was a main effect of sex, $F(1,55)= 18.683$, $p>.000$, $\eta^2= .254$, observed power= .989, such that females (mean=9.682, standard error=.449) had a lower startle response than males (mean=12.443 standard error=.449). There was a significant time x stress x nicotine interaction, $F(1,55)= 5.047$, $p= .029$, $\eta^2= .084$, observed power= .598.

rANCOVA 120 dB with 82 dB pre-pulse, split by sex. See Figure 11. There was a significant nicotine x stress interaction for females at T1, $F(1,27)= 4.661$, $p=.040$, $\eta^2=.147$, observed power=.549.

rANCOVA 120 dB with 82dB pre-pulse, split by sex, and stress. See Figure 11. There was an effect of nicotine approaching significance for females that received WSP at T1, $F(1,13)=4.423$, $p=.055$, $\eta^2=.254$, observed power=.495, such that females that received nicotine (mean=6.074, standard error=1.239) had a lower startle response than females that received saline (mean=9.773, standard error= 1.239). There was a main effect of nicotine for males that did not receive WSP at T1 (see figure 12), $F(1,13)$, $p=.047$, $\eta^2=.270$, observed power=.529, such that males that received nicotine (mean=12.564, standard error=.905) had a greater startle response than males that received saline (mean=9.753, standard error=.905). There were no main effects or significant interactions at T2.

Summary. There was an overall main effect of time and sex, such that startle response at T1 were less than startle responses at T2 and females had lower startle

responses males respectively. There was an overall significant time x stress x nicotine interaction. Males that were not exposed to stress at T1 and received nicotine had a greater startle response than males that were not exposed to stress and received saline.

PERCENT PRE-PULSE INHIBITION.

Percent pre-pulse inhibition was calculated using the equation: (amplitude without PP - amplitude with PP)/amplitude without PP x 100). The percentage was calculated for each ASR with pre-pulse (110 dB with 68 dB pre-pulse, 110 dB with 82 dB pre-pulse, 120 dB with 68 dB pre-pulse, and 120 dB with 82 dB pre-pulse). PPI can be negative or positive because it is based on the increase or decrease of inhibition of startle response with the presence of pre-pulse. Therefore, a negative PPI means that the presence of a pre-pulse had little inhibitory effect and a positive PPI means that the presence of pre-pulse gave an inhibition of startle response (1; 53; 94). An ANCOVA for percent pre-pulse inhibition, using all independent variables, was conducted to determine main effects of and interactions between variables.

Overall rANCOVA *110 dB with 68 dB pre-pulse.* See Figures 13 & 14 and Table 44. There was an effect of sex approaching significance (see figures 13&14), $F(1,55)=2.720$, $p=.055$, $\eta^2=.065$, observed power=.487, such that females (mean= -9.481, standard error=3.257) showed less of an inhibition of startle response with the presence of pre-pulse than males (mean= -.450, standard error=3.257).

ANCOVA *110 dB with 68 dB pre-pulse split by sex*. See Figure 13 and Table 44. There was a main effect of stress for females at T1, $F(1,27)=5.021$, $p=.033$, $\eta^2=.157$, observed power=.580, such that females that received the WSP (mean=-18.652, standard error=5.882) showed little inhibition of startle response with the presence of pre-pulse in

comparison to females that did not receive the WSP (mean=.140, standard error=.140), that showed an inhibition of startle with the presence of pre-pulse. There were no main effects or significant interactions at T2.

ANCOVA *110 dB with 68 dB pre-pulse split by sex and stress.* See Figure 14 and Table 44. There was a main effect of nicotine for males that did not receive the WSP at T1, $F(1,13)=5.337$, $p=.038$, $\eta^2=.291$, observed power=.571, such that males that received nicotine (mean=11.696, standard error=7.016) showed an inhibition of startle response with the presence of pre-pulse in comparison to males that received saline (mean=-11.780, standard error=7.016), that showed little inhibition of startle response with the presence of pre-pulse. There were no main effects or significant interactions at T2.

Summary. The main effect of stress for females at T1 revealed that females that were exposed to the WSP showed little inhibition of startle response, whereas females that did not receive the WSP showed an inhibition of startle response. There was also a main effect of nicotine at T1 for males that were not exposed to stress and received nicotine showed an inhibition of startle response, whereas the rats that received saline showed little inhibition of startle response.

Overall rANCOVA *110 dB with 82 dB pre-pulse.* See Figures 15 & 16 and Table 45. There were no significant main effects or interactions.

ANCOVA *110 dB with 82 dB pre-pulse split by sex.* See Figure 16 and Table 45. There was a main effect of nicotine at T1 for males, $F(1,27) =4.224$, $p=.050$, $\eta^2=.135$, observed power=.509, such that males that received nicotine (mean=8.547, standard error=6.190) showed an inhibition of startle response with the presence of pre-pulse in

comparison to males that received saline (mean=-9.465, standard error=6.190) that showed little inhibition of startle response with the presence of pre-pulse. There were no main effects or significant interactions at T2.

ANCOVA *110 dB with 82 dB pre-pulse split by sex and stress.* See Figure 15 and Tables 40, 41, 42, and 43. There was a main effect of nicotine at T2 for females that did not receive the WSP, $F(1,13)= 5.038$, $p=.043$, $\eta^2=.279$, observed power=.547, such that females that received nicotine (mean=-3.948, standard error=6.052) showed little inhibition of startle response in comparison to females that received saline (mean=15.617, standard error=6.052), that showed an inhibition of startle response with the presence of pre-pulse.

Summary. At T1 males that received nicotine showed an inhibition of startle response with the presence of pre-pulse and males that received saline showed little inhibition of startle response with the presence of pre-pulse. At T2, females that received the WSP and nicotine showed little inhibition of startle response with pre-pulse and females that received the WSP and saline showed little inhibition of startle response with the presence of pre-pulse.

Overall rANCOVA *120 dB with 68 dB pre-pulse.* See Figures 17 & 18 and Table 46. There were no significant main effects or interactions.

ANCOVA *120 dB with 68 dB pre-pulse split by sex.* See Figures 17 & 18 and Table 46. There were no significant main effects or interactions.

ANCOVA *120 dB with 68 dB pre-pulse split by sex and stress.* See Figures 17 & 18 and Table 46. There were no significant main effec0ts or interactions.

Summary. There were no significant main effects or interactions at this level.

Overall rANCOVA *120 dB with 82 dB pre-pulse*. See Figures 19 & 20 and Table 47. There were no significant main effects or interactions.

ANCOVA *120 dB with 82 dB pre-pulse split by sex.* See Figure 19 and Table 47. There was a main effect of stress at T2 for females, $F(1,27)=5.229$, $p=.030$, $\eta^2=.162$, observed power=.597, such that females that received the WSP (mean=4.778, standard error=5,694) showed an inhibition of startle response with the presence of pre-pulse in comparison to females that did not receive the WSP (mean=-13.654, standard error=5.694), that showed little inhibition of startle response with the presence of pre-pulse.

ANCOVA *120 dB with 82 dB pre-pulse split by sex and stress.* There were no significant main effects or interactions.

Summary. At T2, females that received the WSP showed an inhibition of startle response with the presence of pre-pulse, whereas females that were not exposed to the WSP showed little inhibition of startle response with the presence of pre-pulse.

CHAPTER 5: Support of Hypotheses

Specific Aim 1: To determine effects of a military-relevant stressor including threat of an attack by a predator (fox urine) and non-painful unpredictable environmental stimuli in male and female rats.

Hypothesis 1: The hypothesis that stress would deleteriously affect cognition (impaired attention and information processing) as detected by percent pre-pulse inhibition in ASR was **not supported.** PPI revealed a main effect (ME) of stress at T1 for females (for 110 dB with a 68 dB pre-pulse) and a ME of stress at T2 for females (for 120 dB with an 82dB pre-pulse). However, these results revealed opposite effects at each time point and the lack of statistical evidence prevents any firm conclusions. For 110 dB with a 68 dB pre-pulse, females that were exposed to the WSP did not inhibit their startle response compared to females that were not stressed (see figure 13). While at 120 dB with an 82dB pre-pulse, females that were exposed to the WSP startled less compared to females that were not stressed (see figure 19).

Specific Aim 2: To determine whether nicotine alters the effects of stress.

Hypothesis 2: The hypothesis that nicotine will attenuate deleterious effects of stress on cognitive measures (i.e., attenuate deleterious effects of stress on attention, learning, and memory) was **partially supported**. ASR revealed a ME for nicotine for males at T1 for 110 dB with 82dB pre-pulse and at T1 for 120 dB with 82 dB pre-pulse. Stressed males receiving nicotine had lower startle responses than stressed males that received saline (110 dB with 82 dB pre-pulse). Non-stressed males receiving nicotine had greater startles responses than non-stressed males receiving saline (120dB with 82dB pre-pulse). ASR also revealed an effect of nicotine for stressed females at T1

approaching significance (p=.055) at T1 for 110 dB with 82 dB pre-pulse. Stressed females receiving nicotine had lower startle responses than stressed females receiving saline. PPI revealed a ME for nicotine. Non-stressed males receiving nicotine had an inhibited startle response at T1 (for 110 dB with 68 dB prepulse) while non-stressed females receiving nicotine had an inhibited startle response at T2 (for 110 dB with 82 dB prepulse).

Specific Aim 3: To determine if females and males are affected differently by the combination of stress and a licit drugs (nicotine).

Hypothesis 3: The hypothesis that females will be particularly sensitive to effects of stress and to effects of nicotine on stress (i.e., nicotine will be more beneficial for female than for male rats under stress) was **partially supported** because a greater number of significant main effects and interactions were found for females than males. PPI analyses revealed that non-stressed females receiving nicotine showed an inhibited startle response compared to non-stressed females that received saline at T2 (110 dB with 82 dB pre-pulse). PPI analyses showed similar results for males at T1 (110 dB with 68 dB pre-pulse). PPI analyses also revealed that stressed females showed an inhibition of startle response with the presence of pre-pulse in comparison to non-stressed females at T2 (120 dB with 82 dB pre-pulse).

CHAPTER 6: Discussion

STUDY REVIEW

The purpose of this experiment was to examine effects of nicotine and stress on cognitive function through the use of an animal model. Nicotine is the addictive component in tobacco, which is one of the most frequently used substances in the world. While there has been previous research conducted on the effects of nicotine on cognitive function (34; 56; 58), this study utilized a well-established stress paradigm with the inclusion of females. The addition of females was especially important because of the increasing number of women in combat roles, training, and stress (62; 85). The Warrior Stress Paradigm (WSP) utilizes the scent of a predator (synthetic fox urine) and non-pain environmental stimuli (noise, flashing lights, and cage shaking). The WSP is an innovative paradigm that is meant to model combat stress experienced by military personnel. Military personnel have a high prevalence of tobacco use (7)and work in a career attributed with high stress (73; 79). The animal model allowed the researcher to study the effects of nicotine on cognition, in conjunction with a high stress environment. This paradigm also was useful because it is meant to model the stress experienced by military personnel. Military use of nicotine continues to be higher than the general population (7; 82), which can cause impairments (15; 36). The results of this study may be applied to further investigate the effects of nicotine on cognition during deployment.

Three independent variables were manipulated in this experiment, each independent variable had two levels: nicotine (saline, 6 mg/kg nicotine solution); and stress (no warrior stress paradigm, warrior stress paradigm); sex (male, female). The dependent variable was a measure of central information processing and attention (ASR with and without pre-pulse). This experiment included between-subjects and within-

subject comparisons of behavior measured before and after nicotine enhancement and a stressor. The findings of the experiment, general discussion, limitations, and future directions are provided below.

EXPERIMENTAL FINDINGS

Aim 1 of this research study was to determine the effects, specifically cognitive, of a military-relevant stressor in male and female rats. The present experiment used an innovative warrior stress paradigm as a stressor for rats. Previous research has indicated that the use of a warrior stress paradigm increases anxiety-related and depressive-related behaviors in male and female rats (8; 68). However, the cognitive effects of this stress paradigm have not been previously studied. The WSP includes a component of predator stress. In this model the predator stress consists of synthetic fox urine. Previous predator stress paradigms have used the scent of a predator (8; 18; 19; 33)and a live animal (33; 71). Predator stress has been found to be a relevant and accurate stressor because it activates the HPA axis, causes the release of neurotransmitters and messengers (33), activates the immune system (19), and increases the stress hormone in rats (43).

Percent pre-pulse inhibition was used to monitor whether the WSP affected the cognition of the rats. PPI is a calculation, which uses the ASR score of the rat with pre-pulse and without pre-pulse, to determine if the inclusion of the pre-pulse before the acoustic startle stimuli causes an inhibition of the startle. An inhibition of startle response is indicated by a positive percent pre-pulse (PPI), and little or no inhibition is indicated by a negative PPI. The startle response is a natural reaction to sudden, intense stimuli. For this study the startle response was an acoustic stimuli. The startle response can be indicated by a bodily twitch. A pre-pulse is the presentation of a weaker stimulus

preceding the stronger acoustic startle stimuli. PPI provides a measure of sensory gating, which is the ability to filter out, intrusive and unnecessary sensory information (47; 53). Impaired sensory gating abilities have been found in psychiatric populations such as individuals with schizophrenia (5; 53), Huntington's disease (53), obsessive compulsive disorder (94), and attention-deficit disorder (94).

PPI analyses indicated that there was a main effect of stress for the females, but there was no effect of stress for the males. The presence of a pre-pulse did not inhibit the startle response for stressed females at T1 compared to non-stressed females with an acoustic startle stimuli of 110 dB and a pre-pulse of 68 dB. These results indicated that the presence of stress impaired the sensory gating and cognitive ability of the female rats. However, there also was a main effect of stress at T2 for females, for an acoustic startle stimuli of 120 dB and a pre-pulse of 82 dB. Stressed females were able to inhibit their startle response compared to non-stressed females. These results indicated that sensory gating was able to work correctly with the stressed female population at 120 dB with and 82 dB pre-pulse. These results indicated that the cognitive functioning of female rats is impaired with stress and requires a greater stimuli, such as a startling stimulus at a louder decibel level, to work correctly. These findings are consistent with previous research that male and females react differently to stress (10; 23; 66)

Aim 2 of this research study was to determine whether nicotine alters the effects of stress. Previous research has indicated that stress can cause deficits in learning and memory (65; 66) and cognitive function (61). Stress has previously been reported to affect the parts of the brain that are associated with learning and memory (9; 10). However, there are reports of beneficial effects of nicotine on cognition (34; 56; 58).

Therefore, the purpose of this aim was to investigate whether nicotine would be able to attenuate any detrimental cognitive effects of stress on the male and female rats.

The ASR analyses revealed a main effect of nicotine for stressed and non-stressed males and an effect approaching significance (p=.055) for stressed females. Stressed males receiving nicotine had lower startle responses at T1 for an acoustic startle stimuli of 110 dB and a pre-pulse of 82 dB than stressed males receiving saline. Conversely, non-stressed males receiving nicotine had greater startle responses at T1 for an acoustic startle stimuli of 120 dB and a pre-pulse of 82 dB than non-stressed males receiving saline. These results suggest that while nicotine is successful in attenuating deleterious cognitive effects in stressed males it is not successful for non-stressed males. Stressed females receiving nicotine had lower startle responses than the saline group at T1 for an acoustic startle stimuli of 110 dB and a pre-pulse of 82 dB. These results suggest that nicotine is successful in attenuating deleterious cognitive effects in the presence of stress for females.

Aim 3 of this research study was to determine whether females would be particularly sensitive to the effects of stress and to the effects of nicotine on stress. PPI analyses revealed a main effect of nicotine for non-stressed females, a main effect of stress for females, and a main effect of nicotine for non-stressed males. Non-stressed females that received nicotine showed an inhibition of startle response with the presence of pre-pulse at T2 for an acoustic startle stimuli of 110 dB and a pre-pulse of 82 dB pre-pulse. While non-stressed females that received saline at the same time point, startle stimuli, and pre-pulse, showed little inhibition of startle response with the presence of pre-pulse. Stressed females also showed an inhibition of startle response with the

presence of pre-pulse at T2 for an acoustic startle stimuli of 120 dB and a pre-pulse of 82 dB. While non-stressed females that at the same time point, startle stimuli, and pre-pulse, showed little inhibition of startle response with the presence of pre-pulse.

Males showed similar results. PPI analyses revealed that non-stressed males receiving nicotine showed an inhibition of startle response with the presence of pre-pulse at T2 for an acoustic startle stimuli of 110 dB and a pre-pulse of 68 dB. While non-stressed males that received saline showed little inhibition of startle response with the presence of pre-pulse. It should also be noted that PPI analyses indicated a main effect of stress for females, but not for males (see aim 1). The PPI indicates a greater sensitivity to the effects of stress for females. ASR analyses also revealed an overall effect of sex approaching significance for an acoustic startle stimuli of 110 dB and a pre-pulse of 68 dB. Females showed less inhibition of startle response with the presence of pre-pulse than males. These results also indicate a greater sensitivity for females, as there were more main effects of nicotine and stress for females than males.

GENERAL DISCUSSION

The research study was successful in analyzing each of the aims and hypotheses set forth. The purpose of the research study was to study the effects of nicotine and the warrior stress paradigm on cognitive function of male and female rats. Specific areas of interest within the study included the possibility of the attenuating effects of nicotine on cognition, stress having a negative effect on cognition, and sex differences between males in females in the treatment groups. Each of the hypotheses was partially confirmed and the analyses revealed multiple main effects and interactions. The ASR analyses revealed a significant trend in the startle responses, where females consistently had lower startle

responses than males. This finding is consistent with startle response results in both human (76) and animal models (55). Also, while it was hypothesized that females would be more sensitive than males to the effects nicotine on stress, this hypothesis was not fully confirmed. Previous research has indicated that females have a greater sensitivity than males to the effects of nicotine (31; 41; 43). The results revealed an interesting finding within the male population. Males that were exposed to stress had lower startles responses with nicotine. However, males that were not exposed to stress had greater responses with nicotine. This result suggests that nicotine may only be beneficial in assisting with cognitive performance in certain circumstances, such as being in a stressed environment. Overall, the study was successful in addressing each of the aims and hypotheses of interest. It was successful in revealing the possible benefits of nicotine for stressed males, and provided additional support for the sex differences in stress response. However, a main effect of stress was only found in females at different time points. Therefore, further empirical studies are needed to confirm these findings.

LIMITATIONS

It is important to acknowledge the purpose of this study was to provide the researcher with a basis to systematically study the effects of nicotine and predator stress. The primary aim was to assist in the acquisition of knowledge of the effects of nicotine and stress on the mind and body. Because this was an animal model and not a human study, there are limitations with the data that were collected. Although there was a gentling and acclimation period for the rats to allow them to become comfortable with their new surroundings, there was still a chance that the human handling and change of environment caused an increase in the anxiety-related and depressive-related behaviors.

Also, because this was an animal study, the researchers are granted a high level of control that might not otherwise be possible with humans. This results in a decrease of the generalizability of the results to a human population.

Independent Variables. While it was beneficial to examine the effects of nicotine alone because it has previously had beneficial effects (1; 3; 69; 73), this study was meant to model the combat stress experience and most military personnel are using other substances (i.e., caffeine) in conjunction with the nicotine. The combination of these chemicals in the body may possibly cause incredibly different results. The timeline of the research study included the beginning of the stress days immediately following surgery. While the rats were monitored for the possibility of being under too much stress by monitoring food consumption and body weight, it may have been beneficial to have the rats have a day or two between the mini-pump implantation and the start of the warrior stress paradigm. Rats were given a nicotine bitartrate solution through an osmotic mini-pump. While nicotine is the addictive substance in tobacco, it is not the only substance in cigarettes and smokeless tobacco products. By studying the effects of nicotine by itself and not the additional products that are usually in tobacco products it affects the ability for the study to be applied to a human population.

FUTURE DIRECTIONS

Independent Variables. It is worth considering the possibility of studying the effects of nicotine in conjunction with caffeine to better model the human condition in military personnel. Another common stressor within military personnel is sleep deprivation. The addition of this variable may aid in the results of the findings being more applicable to the population of interest (military personnel). The use of a smoke

box, which places the rat into a box and blows tobacco smoke inside, is a possible future direction. While it may be difficult to measure the intake amount, using tobacco smoke instead of solely nicotine would provide the ability to study the effects of tobacco that are usually experienced by a human population. The recent study used an animal model because of the stress manipulation and invasive surgery. However, it would be useful to conduct a similar study with investigate the effects of tobacco within a human population and see if the experiment yields the same results. Although it would not be ethical to manipulate the stress for participants, it is a possibility to use a population that has recently endured a high stress environment, such as recently deployed military personnel. By using participants that are currently smokers it will be possible to investigate the effects of nicotine along with other substances that are in tobacco products.

Dependent Variable. There is an interest in studying the effects of the warrior stress paradigm and nicotine on learning and memory. It would be beneficial to study these cognitive factors with the passive avoidance machine, which is a behavioral measurement of learning and memory. There are also more behavioral measures that can be used to study the effects of nicotine and a stressor on cognition. The radial arm maze tasks the rat to remember the arms it entered to complete the maze (59) and is a measure of working memory (59). The three-panel runway task makes the rat choose between three doors that are held constant throughout the study and is a measure of reference memory (59). The lack of significant findings for effects of stress on cognition as detected by percent pre-pulse inhibition in ASR suggests that it might be beneficial to study effects of a different stress paradigm on cognition.

CHAPTER 7: Summary & Conclusion

This study investigated effects of nicotine and warrior stress, the combination of these two factors, and their effects. This experiment used an animal model on male and female rats to study the effects of nicotine and warrior stress on central information processing and attention. Cognitive functioning of female rats was impaired with stress. However, when a greater stimuli, such as a startling stimulus at a louder decibel level, was used cognition returned to normal. Nicotine was successful in attenuating deleterious cognitive effects in males that were exposed to stress, but had the opposite effect for non-stressed males. The results of the study also revealed that females and males may have different cognitive responses to nicotine, stress, and their interaction. Finally, time was an additional factor in the effect of nicotine on cognition. The longer the nicotine was in the rats (Day 19 vs Day 29), the greater the startle response.

It would be a useful future direction to replicate this study with a human population. The acoustic startle response is a naturalistic response to a sudden stimuli and is measurable in humans as well as the animal model used in this study. In addition there is also a behavioral measurement for startle response for humans (37). Working with a human population would also be beneficial to study the effects of nicotine in combination with other commonly ingested products, such as caffeine to investigate whether the results are consistent with the effects of nicotine alone.

APPENDIX A: ASR Tables

Table 1. Cell Breakdown

	Sex= Female		**Sex= Male**	
	Nicotine= 0 mg/kg	**Nicotine= 6 mg/kg**	**Nicotine= 0 mg/kg**	**Nicotine= 6 mg/kg**
Stress= No WSP	**8**	**8**	**8**	**8**
Stress= WSP	**8**	**8**	**8**	**8**

Table 2. rANCOVA ASR 110 dB with no pre-pulse

Within-Subjects, adjusted values

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Time	.089	1	.089	.013	.911	.000	.051
Time * BL_nopre-pulse_110	3.158	1	3.158	.448	.506	.008	.101
Time * Stress	3.424	1	3.424	.486	.489	.009	.105
Time * Nicotine	.809	1	.809	.115	.736	.002	.063
Time * Sex	16.058	1	16.058	2.278	.137	.040	.317
Time * Stress * Nicotine	35.448	1	35.448	5.028	.029	.084	.596
Time * Stress * Sex	4.318	1	4.318	.612	.437	.011	.120
Time * Nicotine * Sex	.933	1	.933	.132	.717	.002	.065
Time * Stress * Nicotine * Sex	.839	1	.839	.119	.731	.002	.063
Error(Time)	387.766	55	7.050				

Between-Subjects, adjusted values

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Intercept	440.056	1	440.056	25.304	.000	.315	.999
BL_nopre-pulse_110	313.887	1	313.887	18.049	.000	.247	.987
Stress	23.901	1	23.901	1.374	.246	.024	.210
Nicotine	5.901	1	5.901	.339	.563	.006	.088
Sex	333.728	1	333.728	19.190	.000	.259	.990
Stress * Nicotine	42.248	1	42.248	2.429	.125	.042	.334
Stress * Sex	3.948	1	3.948	.227	.636	.004	.075
Nicotine * Sex	8.212	1	8.212	.472	.495	.009	.104
Stress * Nicotine * Sex	.676	1	.676	.039	.844	.001	.054
Error	956.493	55	17.391				

Table 3. rANCOVA ASR 110 dB with 68 dB pre-pulse

Within-Subjects, adjusted values

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Time	11.121	1	11.121	1.201	.278	.021	.190
Time * BL_68_110	4.937	1	4.937	.533	.468	.010	.111
Time * Stress	25.353	1	25.353	2.738	.104	.047	.369
Time * Nicotine	5.675	1	5.675	.613	.437	.011	.120
Time * Sex	2.246	1	2.246	.243	.624	.004	.077
Time * Stress * Nicotine	13.108	1	13.108	1.416	.239	.025	.215
Time * Stress * Sex	2.503	1	2.503	.270	.605	.005	.080
Time * Nicotine * Sex	4.249	1	4.249	.459	.501	.008	.102
Time * Stress * Nicotine * Sex	.973	1	.973	.105	.747	.002	.062
Error(Time)	509.202	55	9.258				

Between-Subjects, adjusted values

Source	Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Intercept	331.731	1	331.731	18.742	.000	.254	.989
BL_68_110	353.109	1	353.109	19.950	.000	.266	.992
Stress	5.682	1	5.682	.321	.573	.006	.086
Nicotine	33.002	1	33.002	1.865	.178	.033	.269
Sex	167.643	1	167.643	9.471	.003	.147	.856
Stress * Nicotine	.280	1	.280	.016	.900	.000	.052
Stress * Sex	.000	1	.000	.000	.998	.000	.050
Nicotine * Sex	11.439	1	11.439	.646	.425	.012	.124
Stress * Nicotine * Sex	1.278	1	1.278	.072	.789	.001	.058
Error	973.489	55	17.700				

Table 4. rANCOVA ASR 110 dB with 82 dB pre-pulse

Within-Subjects, adjusted values

Source	Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Time	2.362	1	2.362	.299	.587	.005	.084
Time * BL_82_110	.005	1	.005	.001	.979	.000	.050
Time * Stress	9.823	1	9.823	1.244	.270	.022	.195
Time * Nicotine	9.909	1	9.909	1.255	.268	.022	.196
Time * Sex	4.062	1	4.062	.514	.476	.009	.109
Time * Stress * Nicotine	22.444	1	22.444	2.842	.098	.049	.381
Time * Stress * Sex	14.061	1	14.061	1.780	.188	.031	.259
Time * Nicotine * Sex	1.033	1	1.033	.131	.719	.002	.065
Time * Stress * Nicotine * Sex	10.197	1	10.197	1.291	.261	.023	.201
Error(Time)	434.419	55	7.899				

Between-Subjects, adjusted values

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Intercept	428.368	1	428.368	20.636	.000	.273	.994
BL_82_110	287.964	1	287.964	13.872	.000	.201	.955
Stress	7.696	1	7.696	.371	.545	.007	.092
Nicotine	.309	1	.309	.015	.903	.000	.052
Sex	204.426	1	204.426	9.848	.003	.152	.869
Stress * Nicotine	11.606	1	11.606	.559	.458	.010	.114
Stress * Sex	.004	1	.004	.000	.989	.000	.050
Nicotine * Sex	.580	1	.580	.028	.868	.001	.053
Stress * Nicotine * Sex	.001	1	.001	.000	.995	.000	.050
Error	1141.713	55	20.758				

Table 5. rANCOVA ASR 120 dB with no pre-pulse

Within-Subjects, adjusted values

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Time	55.168	1	55.168	6.101	.017	.100	.680
Time * BL_nopre-pulse_120	41.945	1	41.945	4.639	.036	.078	.562
Time * Stress	3.949	1	3.949	.437	.512	.008	.100
Time * Nicotine	2.937	1	2.937	.325	.571	.006	.087
Time * Sex	10.224	1	10.224	1.131	.292	.020	.181
Time * Stress * Nicotine	13.272	1	13.272	1.468	.231	.026	.222
Time * Stress * Sex	44.148	1	44.148	4.882	.031	.082	.584
Time * Nicotine * Sex	7.188	1	7.188	.795	.377	.014	.141
Time * Stress * Nicotine * Sex	2.078	1	2.078	.230	.634	.004	.076
Error(Time)	497.348	55	9.043				

Between-Subjects, adjusted values

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Intercept	292.963	1	292.963	19.411	.000	.261	.991
BL_nopre-pulse_120	180.514	1	180.514	11.960	.001	.179	.925
Stress	16.459	1	16.459	1.091	.301	.019	.177
Nicotine	41.926	1	41.926	2.778	.101	.048	.374
Sex	172.213	1	172.213	11.410	.001	.172	.913
Stress * Nicotine	74.861	1	74.861	4.960	.030	.083	.590
Stress * Sex	.361	1	.361	.024	.878	.000	.053
Nicotine * Sex	2.293	1	2.293	.152	.698	.003	.067
Stress * Nicotine * Sex	1.144	1	1.144	.076	.784	.001	.058
Error	830.096	55	15.093				

Table 6. rANCOVA ASR 120 dB with 68 dB pre-pulse

Within-Subjects, adjusted values

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Time	13.502	1	13.502	1.813	.184	.032	.263
Time * BL_68_120	4.833	1	4.833	.649	.424	.012	.124
Time * Stress	2.047	1	2.047	.275	.602	.005	.081
Time * Nicotine	.291	1	.291	.039	.844	.001	.054
Time * Sex	1.071	1	1.071	.144	.706	.003	.066
Time * Stress * Nicotine	35.381	1	35.381	4.752	.034	.080	.572
Time * Stress * Sex	19.271	1	19.271	2.588	.113	.045	.352
Time * Nicotine * Sex	.032	1	.032	.004	.948	.000	.050
Time * Stress * Nicotine * Sex	1.922	1	1.922	.258	.613	.005	.079
Error(Time)	409.514	55	7.446				

Between-Subjects, adjusted values

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Intercept	346.484	1	346.484	25.046	.000	.313	.998
BL_68_120	368.856	1	368.856	26.663	.000	.326	.999
Stress	25.324	1	25.324	1.831	.182	.032	.265
Nicotine	7.508	1	7.508	.543	.464	.010	.112
Sex	411.112	1	411.112	29.717	.000	.351	1.000
Stress * Nicotine	.073	1	.073	.005	.942	.000	.051
Stress * Sex	.281	1	.281	.020	.887	.000	.052
Nicotine * Sex	.950	1	.950	.069	.794	.001	.058
Stress * Nicotine * Sex	.010	1	.010	.001	.979	.000	.050
Error	760.874	55	13.834				

Table 7. rANCOVA ASR 120 dB with 82 dB pre-pulse

Within-Subjects, adjusted values

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Time	53.025	1	53.025	7.250	.009	.116	.753
Time * BL_82_120	28.130	1	28.130	3.846	.055	.065	.487
Time * Stress	10.769	1	10.769	1.473	.230	.026	.222
Time * Nicotine	1.859	1	1.859	.254	.616	.005	.079
Time * Sex	6.672	1	6.672	.912	.344	.016	.155
Time * Stress * Nicotine	36.909	1	36.909	5.047	.029	.084	.598
Time * Stress * Sex	27.178	1	27.178	3.716	.059	.063	.474
Time * Nicotine * Sex	6.966	1	6.966	.953	.333	.017	.160
Time * Stress * Nicotine * Sex	.243	1	.243	.033	.856	.001	.054
Error(Time)	402.232	55	7.313				

Between-Subjects, adjusted values

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Intercept	471.193	1	471.193	37.062	.000	.403	1.000
BL_82_120	322.196	1	322.196	25.343	.000	.315	.999
Stress	21.043	1	21.043	1.655	.204	.029	.244
Nicotine	8.163	1	8.163	.642	.426	.012	.123
Sex	237.521	1	237.521	18.683	.000	.254	.989
Stress * Nicotine	25.734	1	25.734	2.024	.160	.035	.287
Stress * Sex	9.896	1	9.896	.778	.381	.014	.140
Nicotine * Sex	14.944	1	14.944	1.175	.283	.021	.187
Stress * Nicotine * Sex	8.950	1	8.950	.704	.405	.013	.131
Error	699.245	55	12.714				

Table 8. rANCOVA ASR 110 dB with no pre-pulse, Females

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	2.230	1	2.230	.326	.573	.012	.085
Time * BL_noprepulse_110	4.545	1	4.545	.665	.422	.024	.123
Time * Stress	.031	1	.031	.004	.947	.000	.050
Time * Nicotine	.121	1	.121	.018	.895	.001	.052
Time * Stress * Nicotine	24.549	1	24.549	3.590	.069	.117	.447
Error(Time)	184.634	27	6.838				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	121.372	1	121.372	6.205	.019	.187	.671
BL_noprepulse_110	174.631	1	174.631	8.928	.006	.248	.821
Stress	23.607	1	23.607	1.207	.282	.043	.185
Nicotine	.164	1	.164	.008	.928	.000	.051
Stress * Nicotine	14.483	1	14.483	.740	.397	.027	.132
Error	528.129	27	19.560				

Table 9. rANCOVA ASR 110 dB with no pre-pulse, Males

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	3.638	1	3.638	.487	.491	.018	.103
Time * BL_noprepulse_110	.099	1	.099	.013	.909	.000	.051
Time * Stress	7.902	1	7.902	1.058	.313	.038	.168
Time * Nicotine	1.567	1	1.567	.210	.651	.008	.073
Time * Stress * Nicotine	12.836	1	12.836	1.719	.201	.060	.244
Error(Time)	201.647	27	7.468				

Between Subjects

Source	Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	334.868	1	334.868	21.112	.000	.439	.993
BL_noprepulse_110	139.365	1	139.365	8.786	.006	.246	.815
Stress	4.248	1	4.248	.268	.609	.010	.079
Nicotine	14.283	1	14.283	.900	.351	.032	.150
Stress * Nicotine	28.017	1	28.017	1.766	.195	.061	.249
Error	428.256	27	15.861				

Table 10. rANCOVA ASR 110 dB with 68 dB pre-pulse, Females

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	.002	1	.002	.000	.987	.000	.050
Time * BL_68_110	.336	1	.336	.046	.832	.002	.055
Time * Stress	4.608	1	4.608	.631	.434	.023	.119
Time * Nicotine	.133	1	.133	.018	.894	.001	.052
Time * Stress * Nicotine	16.325	1	16.325	2.235	.147	.076	.303
Error(Time)	197.252	27	7.306				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	64.342	1	64.342	2.846	.103	.095	.370
BL_68_110	268.153	1	268.153	11.859	.002	.305	.913
Stress	1.818	1	1.818	.080	.779	.003	.059
Nicotine	.769	1	.769	.034	.855	.001	.054
Stress * Nicotine	5.398	1	5.398	.239	.629	.009	.076
Error	610.517	27	22.612				

Table 11. rANCOVA ASR 110 dB with 68 dB pre-pulse, Males

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	22.940	1	22.940	2.053	.163	.071	.282
Time * BL_68_110	14.833	1	14.833	1.327	.259	.047	.199
Time * Stress	19.428	1	19.428	1.739	.198	.060	.246
Time * Nicotine	4.553	1	4.553	.407	.529	.015	.094
Time * Stress * Nicotine	2.702	1	2.702	.242	.627	.009	.076
Error(Time)	301.719	27	11.175				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	312.705	1	312.705	24.303	.000	.474	.997
BL_68_110	100.527	1	100.527	7.813	.009	.224	.769
Stress	1.843	1	1.843	.143	.708	.005	.065
Nicotine	25.604	1	25.604	1.990	.170	.069	.275
Stress * Nicotine	.671	1	.671	.052	.821	.002	.056
Error	347.401	27	12.867				

Table 12 . rANCOVA ASR 110 dB with 82 dB pre-pulse, Females

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	3.681	1	3.681	.483	.493	.018	.103
Time * BL_82_110	6.974	1	6.974	.915	.347	.033	.152
Time * Stress	.085	1	.085	.011	.917	.000	.051
Time * Nicotine	.040	1	.040	.005	.943	.000	.051
Time * Stress * Nicotine	5.389	1	5.389	.707	.408	.026	.128
Error(Time)	205.909	27	7.626				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	27.751	1	27.751	1.386	.249	.049	.206
BL_82_110	236.983	1	236.983	11.837	.002	.305	.912
Stress	4.841	1	4.841	.242	.627	.009	.076
Nicotine	2.159	1	2.159	.108	.745	.004	.062
Stress * Nicotine	.154	1	.154	.008	.931	.000	.051
Error	540.545	27	20.020				

Table 13. rANCOVA ASR 110 dB with 82 dB pre-pulse, Males

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	11.998	1	11.998	1.486	.233	.052	.217
Time * BL_82_110	3.513	1	3.513	.435	.515	.016	.098
Time * Stress	21.846	1	21.846	2.705	.112	.091	.355
Time * Nicotine	7.587	1	7.587	.939	.341	.034	.155
Time * Stress * Nicotine	30.629	1	30.629	3.793	.062	.123	.467
Error(Time)	218.028	27	8.075				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	516.413	1	516.413	24.982	.000	.481	.998
BL_82_110	94.017	1	94.017	4.548	.042	.144	.538
Stress	2.678	1	2.678	.130	.722	.005	.064
Nicotine	.366	1	.366	.018	.895	.001	.052
Stress * Nicotine	8.767	1	8.767	.424	.520	.015	.096
Error	558.131	27	20.672				

Table 14. rANCOVA ASR 120 dB with no pre-pulse, Females

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	.000	1	.000	.000	.994	.000	.050
Time * BL_noprepulse_120	.398	1	.398	.050	.824	.002	.055
Time * Stress	15.236	1	15.236	1.925	.177	.067	.268
Time * Nicotine	2.309	1	2.309	.292	.594	.011	.082
Time * Stress * Nicotine	14.476	1	14.476	1.829	.187	.063	.257
Error(Time)	213.667	27	7.914				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	56.572	1	56.572	4.168	.051	.134	.504
BL_noprepulse_120	112.482	1	112.482	8.287	.008	.235	.792
Stress	4.580	1	4.580	.337	.566	.012	.087
Nicotine	6.584	1	6.584	.485	.492	.018	.103
Stress * Nicotine	25.417	1	25.417	1.873	.182	.065	.262
Error	366.476	27	13.573				

Table 15. rANCOVA ASR 120 dB with no pre-pulse, Males

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	91.593	1	91.593	10.023	.004	.271	.862
Time * BL_noprepulse_120	78.496	1	78.496	8.590	.007	.241	.807
Time * Stress	36.521	1	36.521	3.996	.056	.129	.487
Time * Nicotine	4.476	1	4.476	.490	.490	.018	.104
Time * Stress * Nicotine	13.184	1	13.184	1.443	.240	.051	.212
Error(Time)	246.732	27	9.138				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	262.339	1	262.339	15.530	.001	.365	.967
BL_noprepulse_120	75.569	1	75.569	4.474	.044	.142	.532
Stress	10.659	1	10.659	.631	.434	.023	.120
Nicotine	22.397	1	22.397	1.326	.260	.047	.199
Stress * Nicotine	31.485	1	31.485	1.864	.183	.065	.261
Error	456.083	27	16.892				

Table 16. rANCOVA ASR 120 dB with 68 dB pre-pulse, Females

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	4.643	1	4.643	.681	.417	.025	.125
Time * BL_68_120	1.519	1	1.519	.223	.641	.008	.074
Time * Stress	4.405	1	4.405	.646	.429	.023	.121
Time * Nicotine	.023	1	.023	.003	.954	.000	.050
Time * Stress * Nicotine	9.369	1	9.369	1.374	.251	.048	.205
Error(Time)	184.095	27	6.818				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	100.801	1	100.801	6.157	.020	.186	.667
BL_68_120	157.914	1	157.914	9.645	.004	.263	.849
Stress	10.198	1	10.198	.623	.437	.023	.119
Nicotine	7.907	1	7.907	.483	.493	.018	.103
Stress * Nicotine	.426	1	.426	.026	.873	.001	.053
Error	442.068	27	16.373				

Table 17. rANCOVA ASR 120 dB with 68 dB pre-pulse, Males

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	9.193	1	9.193	1.102	.303	.039	.173
Time * BL_68_120	3.506	1	3.506	.420	.522	.015	.096
Time * Stress	16.090	1	16.090	1.929	.176	.067	.268
Time * Nicotine	.259	1	.259	.031	.861	.001	.053
Time * Stress * Nicotine	30.300	1	30.300	3.632	.067	.119	.452
Error(Time)	225.227	27	8.342				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power[b]
Intercept	258.224	1	258.224	21.985	.000	21.985	.995
BL_68_120	212.626	1	212.626	18.103	.000	18.103	.984
Stress	16.396	1	16.396	1.396	.248	1.396	.207
Nicotine	1.628	1	1.628	.139	.713	.139	.065
Stress * Nicotine	.024	1	.024	.002	.964	.002	.050
Error	317.122	27	11.745				

Table 18. rANCOVA ASR 120 dB with 82 dB pre-pulse, Females

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	5.957	1	5.957	.988	.329	.035	.160
Time * BL_82_120	2.038	1	2.038	.338	.566	.012	.087
Time * Stress	2.334	1	2.334	.387	.539	.014	.092
Time * Nicotine	4.640	1	4.640	.769	.388	.028	.135
Time * Stress * Nicotine	25.746	1	25.746	4.270	.049	.137	.513
Error(RM82with120)	162.814	27	6.030				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	71.651	1	71.651	5.592	.025	.172	.626
BL_82_120	165.211	1	165.211	12.893	.001	.323	.933
Stress	27.445	1	27.445	2.142	.155	.073	.292
Nicotine	2.280	1	2.280	.178	.676	.007	.069
Stress * Nicotine	18.429	1	18.429	1.438	.241	.051	.212
Error	345.976	27	12.814				

Table 19. rANCOVA ASR 120 dB with 82 dB pre-pulse, Males

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	53.678	1	53.678	6.168	.020	.186	.668
Time * BL_82_120	30.549	1	30.549	3.511	.072	.115	.439
Time * Stress	35.310	1	35.310	4.058	.054	.131	.493
Time * Nicotine	1.390	1	1.390	.160	.693	.006	.067
Time * Stress * Nicotine	15.230	1	15.230	1.750	.197	.061	.248
Error(RM82with120)	234.960	27	8.702				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	472.278	1	472.278	36.912	.000	.578	1.000
BL_82_120	164.793	1	164.793	12.880	.001	.323	.933
Stress	.858	1	.858	.067	.798	.002	.057
Nicotine	19.792	1	19.792	1.547	.224	.054	.224
Stress * Nicotine	3.250	1	3.250	.254	.618	.009	.077
Error	345.461	27	12.795				

Table 20. rANCOVA ASR 110 dB with no pre-pulse, Females, Stress

Within Subjects

Source	Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	2.202	1	2.202	.502	.491	.037	.101
Time * BL_noprepulse_110	4.017	1	4.017	.915	.356	.066	.144
Time * Nicotine	11.831	1	11.831	2.696	.125	.172	.331
Error(Time)	57.050	13	4.388				

Between Subjects

Source	Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	82.895	1	82.895	2.593	.131	.166	.320
BL_noprepulse_110	48.415	1	48.415	1.514	.240	.104	.207
Nicotine	9.608	1	9.608	.301	.593	.023	.080
Error	415.582	13	31.968				

Table 21. rANCOVA ASR 110 dB with no pre-pulse, Females, No Stress

Within Subjects

Source	Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	.379	1	.379	.039	.847	.003	.054
Time * BL_noprepulse_110	1.000	1	1.000	.102	.754	.008	.060
Time * Nicotine	8.482	1	8.482	.867	.369	.063	.139
Error(Time)	127.112	13	9.778				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	40.737	1	40.737	5.300	.039	.290	.568
BL_noprepulse_110	138.843	1	138.843	18.064	.001	.582	.975
Nicotine	.299	1	.299	.039	.847	.003	.054
Error	99.920	13	7.686				

Table 22. rANCOVA ASR 110 dB with no pre-pulse, Males, Stress

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	.354	1	.354	.045	.836	.003	.054
Time * BL_noprepulse_110	2.339	1	2.339	.296	.596	.022	.080
Time * Nicotine	13.058	1	13.058	1.650	.221	.113	.222
Error(Time)	102.876	13	7.914				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	141.503	1	141.503	6.725	.022	.341	.670
BL_noprepulse_110	48.287	1	48.287	2.295	.154	.150	.290
Nicotine	1.303	1	1.303	.062	.807	.005	.056
Error	273.522	13	21.040				

Table 23. rANCOVA ASR 110 dB with no pre-pulse, Males, No Stress

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	9.727	1	9.727	1.320	.271	.092	.187
Time * BL_noprepulse_110	.707	1	.707	.096	.762	.007	.060
Time * Nicotine	2.820	1	2.820	.383	.547	.029	.089
Error(Time)	95.823	13	7.371				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	195.444	1	195.444	16.466	.001	.559	.963
BL_noprepulse_110	91.512	1	91.512	7.710	.016	.372	.728
Nicotine	41.544	1	41.544	3.500	.084	.212	.410
Error	154.300	13	11.869				

Table 24. rANCOVA ASR 110 dB with 68 dB pre-pulse, Females, Stress

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	.127	1	.127	.022	.884	.002	.052
Time * BL_68_110	.216	1	.216	.037	.850	.003	.054
Time * Nicotine	6.997	1	6.997	1.214	.291	.085	.176
Error(Time)	74.932	13	5.764				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	47.103	1	47.103	1.630	.224	.111	.220
BL_68_110	145.960	1	145.960	5.051	.043	.280	.548
Nicotine	4.954	1	4.954	.171	.686	.013	.067
Error	375.646	13	28.896				

Table 25. rANCOVA ASR 110 dB with 68 dB pre-pulse, Females, No Stress

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	.488	1	.488	.053	.822	.004	.055
Time * BL_68_110	2.332	1	2.332	.252	.624	.019	.075
Time * Nicotine	11.523	1	11.523	1.247	.284	.088	.179
Error(Time)	120.107	13	9.239				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	16.999	1	16.999	.947	.348	.068	.147
BL_68_110	123.819	1	123.819	6.901	.021	.347	.681
Nicotine	2.476	1	2.476	.138	.716	.011	.064
Error	233.244	13	17.942				

Table 26. rANCOVA ASR 110 dB with 68 dB pre-pulse, Males, Stress

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	1.726	1	1.726	.113	.742	.009	.061
Time * BL_68_110	2.426	1	2.426	.159	.697	.012	.066
Time * Nicotine	8.814	1	8.814	.578	.461	.043	.109
Error(Time)	198.402	13	15.262				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	89.383	1	89.383	9.749	.008	.429	.823
BL_68_110	81.215	1	81.215	8.858	.011	.405	.786
Nicotine	15.186	1	15.186	1.656	.221	.113	.222
Error	119.192	13	9.169				

Table 27. rANCOVA ASR 110 dB with 68 dB pre-pulse, Males, No Stress

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	30.216	1	30.216	3.884	.070	.230	.446
Time * BL_68_110	14.597	1	14.597	1.876	.194	.126	.246
Time * Nicotine	.037	1	.037	.005	.946	.000	.050
Error(Time)	101.127	13	7.779				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	239.134	1	239.134	14.162	.002	.521	.935
BL_68_110	28.003	1	28.003	1.658	.220	.113	.223
Nicotine	14.598	1	14.598	.864	.369	.062	.139
Error	219.517	13	16.886				

Table 28. rANCOVA ASR 110 dB with 82 dB pre-pulse, Females, Stress

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	7.412	1	7.412	.814	.383	.059	.133
Time * BL_82_110	11.717	1	11.717	1.286	.277	.090	.183
Time * Nicotine	4.513	1	4.513	.495	.494	.037	.100
Error(Time)	118.410	13	9.108				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	5.515	1	5.515	.223	.645	.017	.072
BL_82_110	163.234	1	163.234	6.596	.023	.337	.661
Nicotine	.589	1	.589	.024	.880	.002	.052
Error	321.738	13	24.749				

Table 29. rANCOVA ASR 110 dB with 82 dB pre-pulse, Females, No Stress

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	.062	1	.062	.010	.923	.001	.051
Time * BL_82_110	.007	1	.007	.001	.974	.000	.050
Time * Nicotine	.010	1	.010	.002	.969	.000	.050
Error(Time)	82.750	13	6.365				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	27.138	1	27.138	1.635	.223	.112	.220
BL_82_110	76.791	1	76.791	4.627	.051	.262	.512
Nicotine	.000	1	.000	.000	.997	.000	.050
Error	215.765	13	16.597				

Table 30. rANCOVA ASR 110 dB with 82 dB pre-pulse, Males, Stress

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	11.370	1	11.370	1.562	.233	.107	.212
Time * BL_82_110	12.512	1	12.512	1.719	.213	.117	.229
Time * Nicotine	28.264	1	28.264	3.883	.070	.230	.446
Error(Time)	94.621	13	7.279				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	226.004	1	226.004	12.310	.004	.486	.900
BL_82_110	68.453	1	68.453	3.728	.076	.223	.432
Nicotine	5.129	1	5.129	.279	.606	.021	.078
Error	238.675	13	18.360				

Table 31. rANCOVA ASR 110 dB with 82 dB pre-pulse, Males, No Stress

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	1.441	1	1.441	.166	.691	.013	.067
Time * BL_82_110	1.342	1	1.342	.154	.701	.012	.065
Time * Nicotine	3.903	1	3.903	.449	.515	.033	.095
Error(Time)	113.065	13	8.697				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	293.665	1	293.665	12.040	.004	.481	.893
BL_82_110	27.951	1	27.951	1.146	.304	.081	.168
Nicotine	2.807	1	2.807	.115	.740	.009	.061
Error	317.069	13	24.390				

Table 32. rANCOVA ASR 120 dB with no dB pre-pulse, Females, Stress

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	5.275	1	5.275	.820	.382	.059	.134
Time * BL_noprepulse_120	1.088	1	1.088	.169	.688	.013	.067
Time * Nicotine	14.767	1	14.767	2.295	.154	.150	.290
Error(Time)	83.633	13	6.433				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	64.469	1	64.469	4.262	.060	.247	.481
BL_noprepulse_120	42.512	1	42.512	2.811	.118	.178	.342
Nicotine	6.991	1	6.991	.462	.509	.034	.097
Error	196.633	13	15.126				

Table 33. rANCOVA ASR 120 dB with no dB pre-pulse, Females, No Stress

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	7.475	1	7.475	.794	.389	.058	.131
Time * BL_noprepulse_120	6.913	1	6.913	.734	.407	.053	.125
Time * Nicotine	9.133	1	9.133	.970	.343	.069	.150
Error(Time)	122.431	13	9.418				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	1.662	1	1.662	.140	.715	.011	.064
BL_noprepulse_120	85.271	1	85.271	7.173	.019	.356	.697
Nicotine	2.218	1	2.218	.187	.673	.014	.069
Error	154.543	13	11.888				

Table 34. rANCOVA ASR 120 dB with no dB pre-pulse, Males, Stress

Within Subject

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	92.336	1	92.336	10.659	.006	.451	.855
Time * BL_noprepulse_120	103.171	1	103.171	11.910	.004	.478	.890
Time * Nicotine	1.165	1	1.165	.134	.720	.010	.063
Error(Time)	112.614	13	8.663				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	102.444	1	102.444	4.753	.048	.268	.523
BL_noprepulse_120	23.378	1	23.378	1.085	.317	.077	.162
Nicotine	.342	1	.342	.016	.902	.001	.052
Error	280.194	13	21.553				

Table 35. rANCOVA ASR 120 dB with no dB pre-pulse, Males, No Stress

Within Subject

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	25.696	1	25.696	3.382	.089	.206	.399
Time * BL_noprepulse_120	10.665	1	10.665	1.404	.257	.097	.196
Time * Nicotine	8.071	1	8.071	1.062	.322	.076	.159
Error(Time)	98.778	13	7.598				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	165.595	1	165.595	12.274	.004	.486	.899
BL_noprepulse_120	52.690	1	52.690	3.905	.070	.231	.448
Nicotine	53.608	1	53.608	3.973	.068	.234	.455
Error	175.389	13	13.491				

Table 36. rANCOVA ASR 120 dB with 68 dB pre-pulse, Females, Stress

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	.007	1	.007	.003	.961	.000	.050
Time * BL_68_120	1.365	1	1.365	.485	.498	.036	.099
Time * Nicotine	11.226	1	11.226	3.992	.067	.235	.456
Error(Time)	36.559	13	2.812				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	7.110	1	7.110	.387	.544	.029	.089
BL_68_120	183.608	1	183.608	10.005	.007	.435	.832
Nicotine	8.701	1	8.701	.474	.503	.035	.098
Error	238.578	13	18.352				

Table 37. rANCOVA ASR 120 dB with 68 dB pre-pulse, Females, No Stress

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	10.393	1	10.393	.980	.340	.070	.151
Time * BL_68_120	9.775	1	9.775	.921	.355	.066	.145
Time * Nicotine	.001	1	.001	.000	.994	.000	.050
Error(Time)	137.916	13	10.609				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	141.862	1	141.862	11.267	.005	.464	.873
BL_68_120	14.115	1	14.115	1.121	.309	.079	.166
Nicotine	27.512	1	27.512	2.185	.163	.144	.278
Error	163.681	13	12.591				

Table 38. rANCOVA ASR 120 dB with 68 dB pre-pulse, Males, Stress

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	.593	1	.593	.061	.809	.005	.056
Time * BL_68_120	.667	1	.667	.069	.797	.005	.057
Time * Nicotine	18.504	1	18.504	1.904	.191	.128	.249
Error(Time)	126.323	13	9.717				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	35.182	1	35.182	4.180	.062	.243	.473
BL_68_120	179.581	1	179.581	21.335	.000	.621	.989
Nicotine	1.881	1	1.881	.223	.644	.017	.072
Error	109.423	13	8.417				

Table 39 . rANCOVA ASR 120 dB with 68 dB pre-pulse, Males, No Stress

Within Subject

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	13.294	1	13.294	1.753	.208	.119	.233
Time * BL_68_120	3.173	1	3.173	.418	.529	.031	.092
Time * Nicotine	12.269	1	12.269	1.618	.226	.111	.218
Error(Time)	98.571	13	7.582				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	275.197	1	275.197	19.518	.001	.600	.983
BL_68_120	57.449	1	57.449	4.074	.065	.239	.464
Nicotine	1.887	1	1.887	.134	.720	.010	.063
Error	183.295	13	14.100				

Table 40. rANCOVA ASR 120 dB with 82 dB pre-pulse, Females, Stress

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	.812	1	.812	.272	.611	.020	.077
Time * BL_82_120	4.317	1	4.317	1.446	.251	.100	.200
Time * Nicotine	35.385	1	35.385	11.851	.004	.477	.889
Error(Time)	38.816	13	2.986				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	22.327	1	22.327	1.244	.285	.087	.179
BL_82_120	85.712	1	85.712	4.774	.048	.269	.525
Nicotine	19.610	1	19.610	1.092	.315	.078	.163
Error	233.393	13	17.953				

Table 41. rANCOVA ASR 120 dB with 82 dB pre-pulse, Females, No Stress

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	18.326	1	18.326	2.255	.157	.148	.285
Time * BL_82_120	16.066	1	16.066	1.977	.183	.132	.256
Time * Nicotine	.006	1	.006	.001	.979	.000	.050
Error(Time)	105.655	13	8.127				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	52.081	1	52.081	6.023	.029	.317	.622
BL_82_120	79.677	1	79.677	9.215	.010	.415	.801
Nicotine	3.656	1	3.656	.423	.527	.032	.093
Error	112.405	13	8.647				

Table 42. rANCOVA ASR 120 dB with 82 dB pre-pulse, Males, Stress

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	34.649	1	34.649	3.423	.087	.208	.403
Time * BL_82_120	37.403	1	37.403	3.695	.077	.221	.429
Time * Nicotine	.658	1	.658	.065	.803	.005	.056
Error(Time)	131.591	13	10.122				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	121.728	1	121.728	7.164	.019	.355	.697
BL_82_120	94.352	1	94.352	5.553	.035	.299	.587
Nicotine	6.213	1	6.213	.366	.556	.027	.087
Error	220.884	13	16.991				

Table 43. rANCOVA ASR 120 dB with 82 dB pre-pulse, Males, No Stress

Within Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Time	30.020	1	30.020	4.278	.059	.248	.482
Time * BL_82_120	5.296	1	5.296	.755	.401	.055	.127
Time * Nicotine	12.456	1	12.456	1.775	.206	.120	.235
Error(Time)	91.220	13	7.017				

Between Subjects

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[b]
Intercept	359.845	1	359.845	39.471	.000	.752	1.000
BL_82_120	76.502	1	76.502	8.391	.012	.392	.764
Nicotine	19.474	1	19.474	2.136	.168	.141	.273
Error	118.517	13	9.117				

APPENDIX B: ASR Figures

Figure 1. 110 dB with no pre-pulse, Females

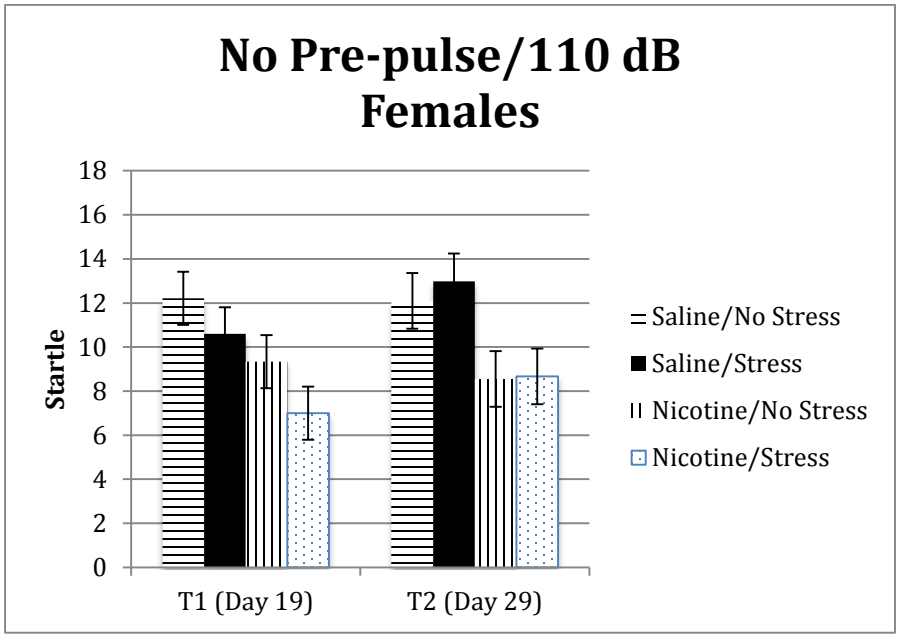


Figure 2. 110 dB with no pre-pulse, Males

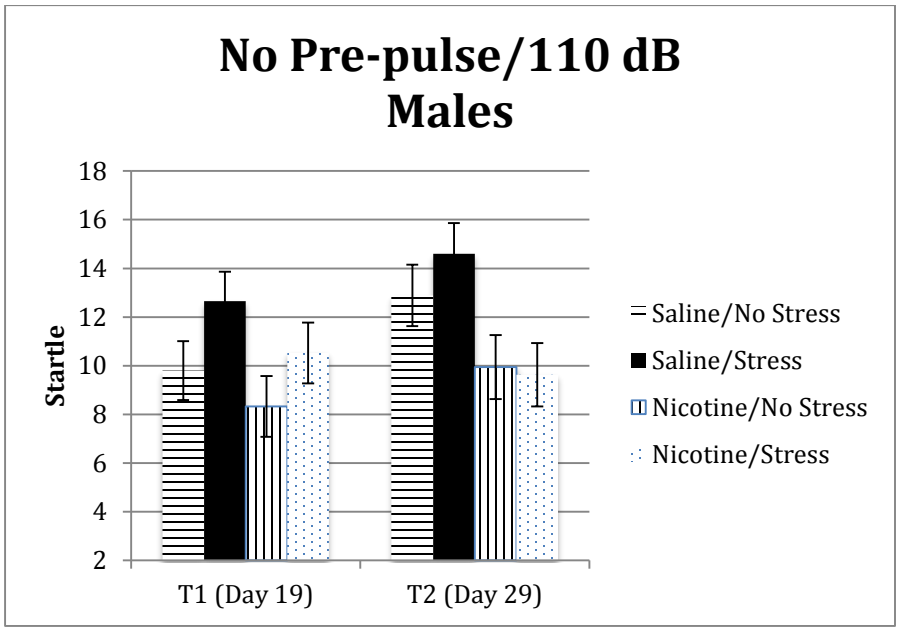


Figure 3. 110 dB with 68 dB pre-pulse, Females

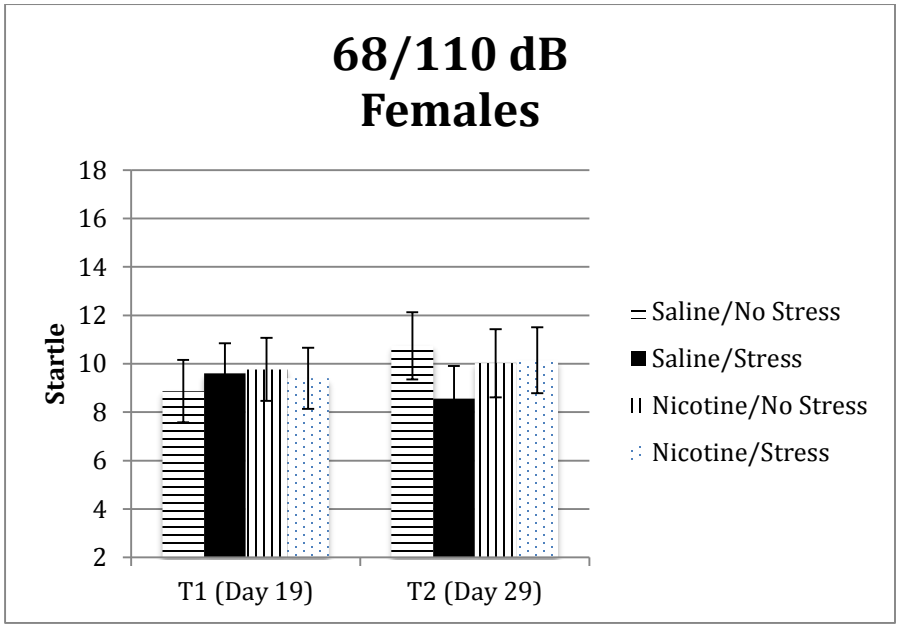


Figure 4. 110 dB with 68 dB pre-pulse, Males

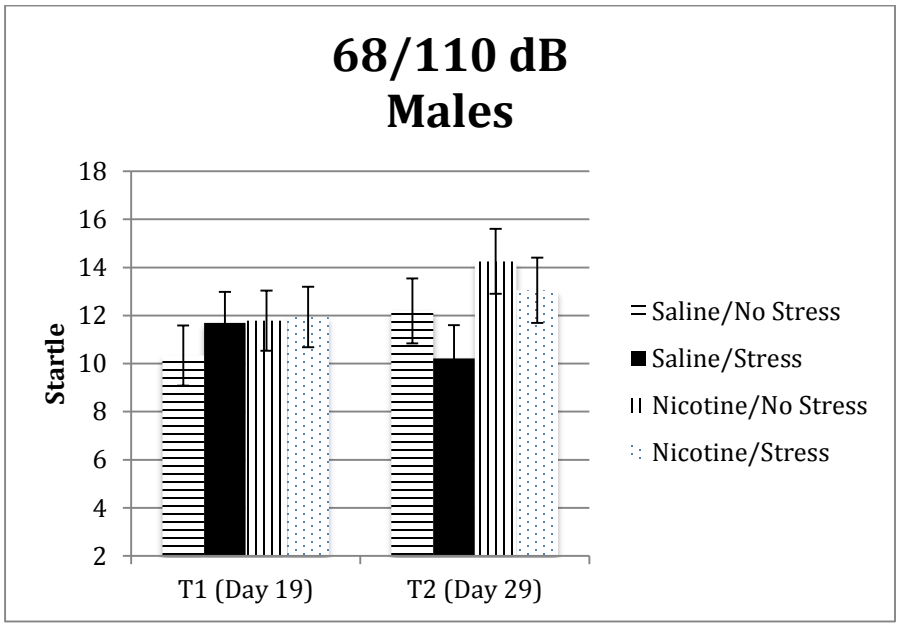


Figure 5. 110 dB with 82 dB pre-pulse, Females

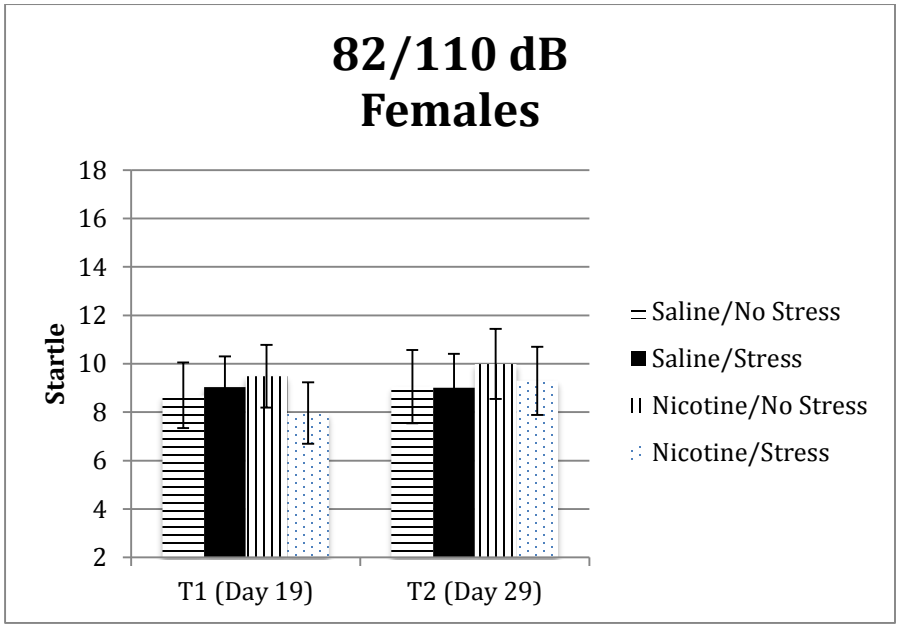


Figure 6. 110 dB with 82 dB pre-pulse, Males

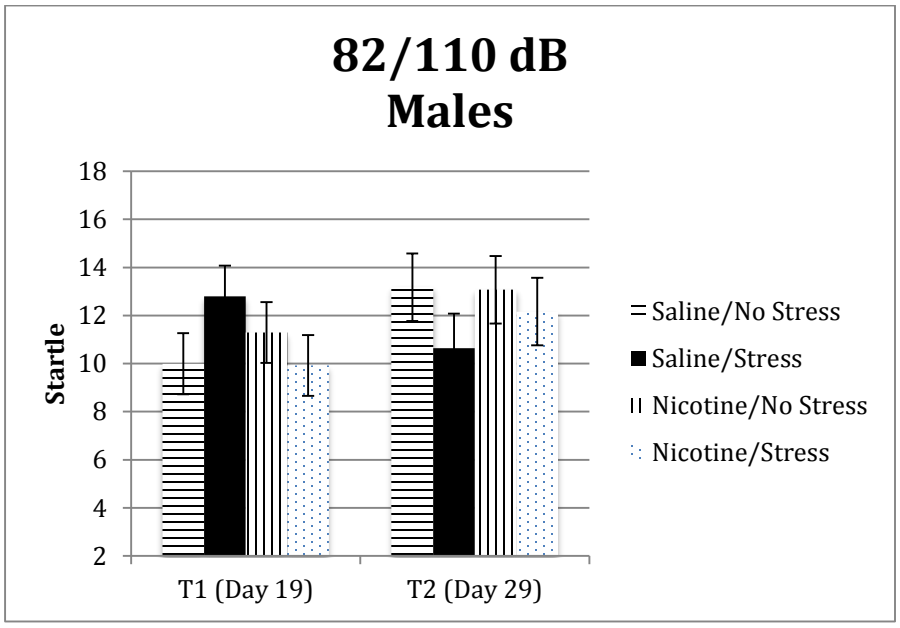


Figure 7. 120 dB with no pre-pulse, Females

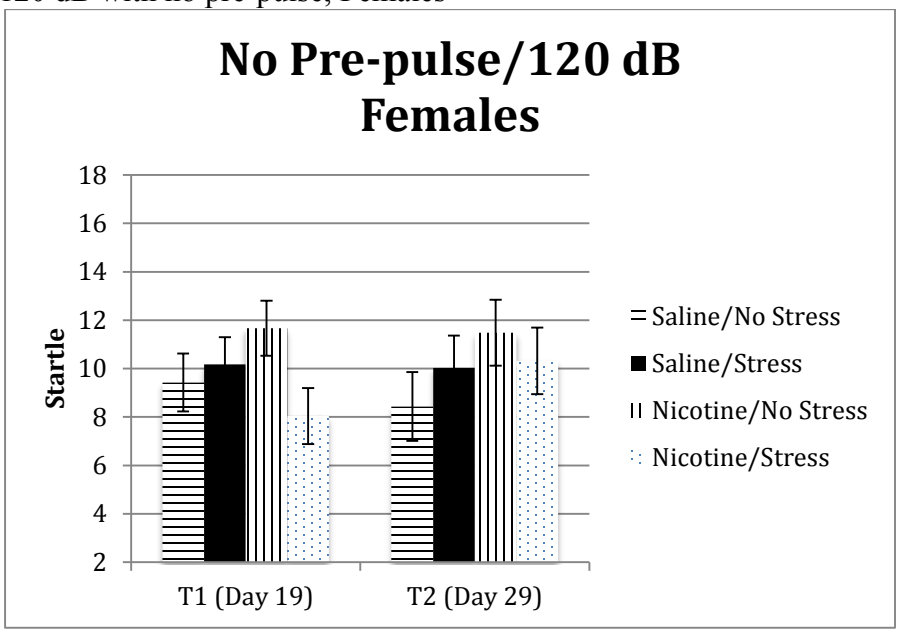


Figure 8. 120 dB with no pre-pulse, Males

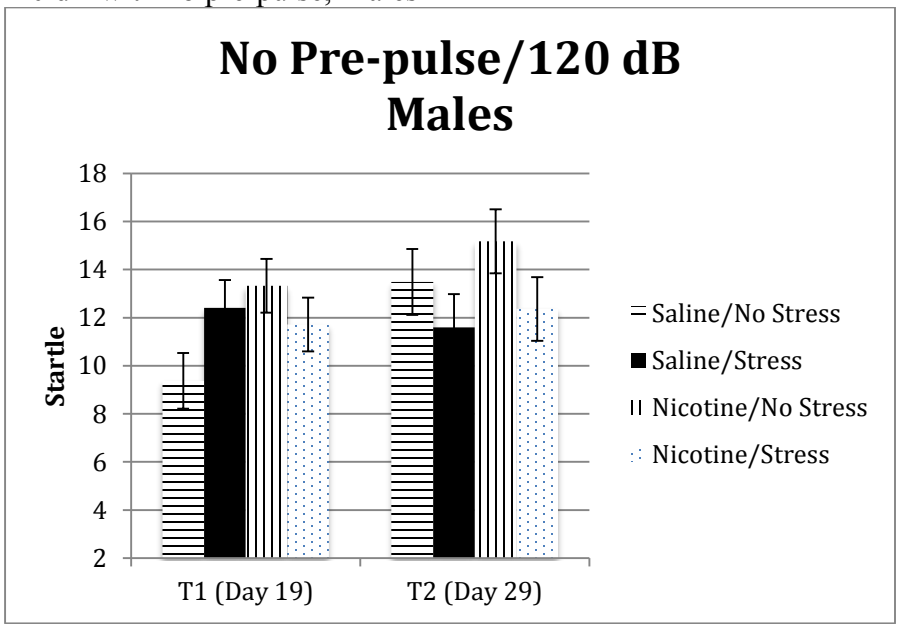


Figure 9. 120 dB with 68 pre-pulse, Females

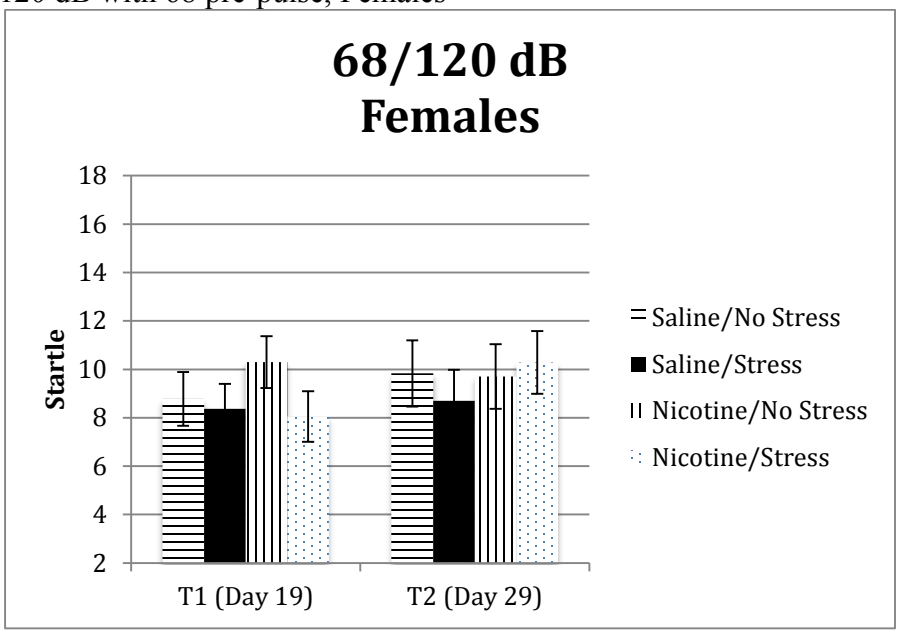


Figure 10. 120 dB with 68 pre-pulse, Males

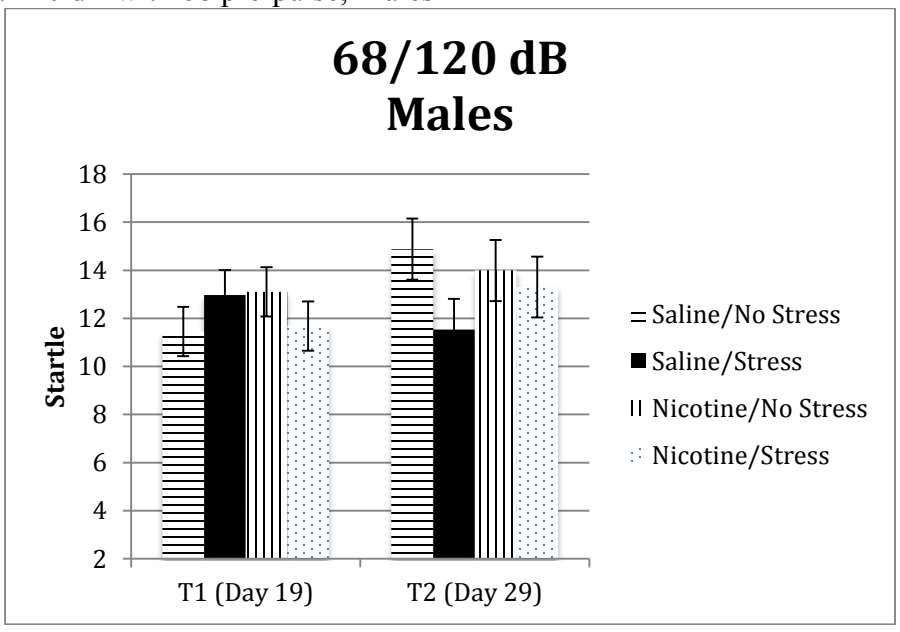


Figure 11. 120 dB with 82 pre-pulse, Females

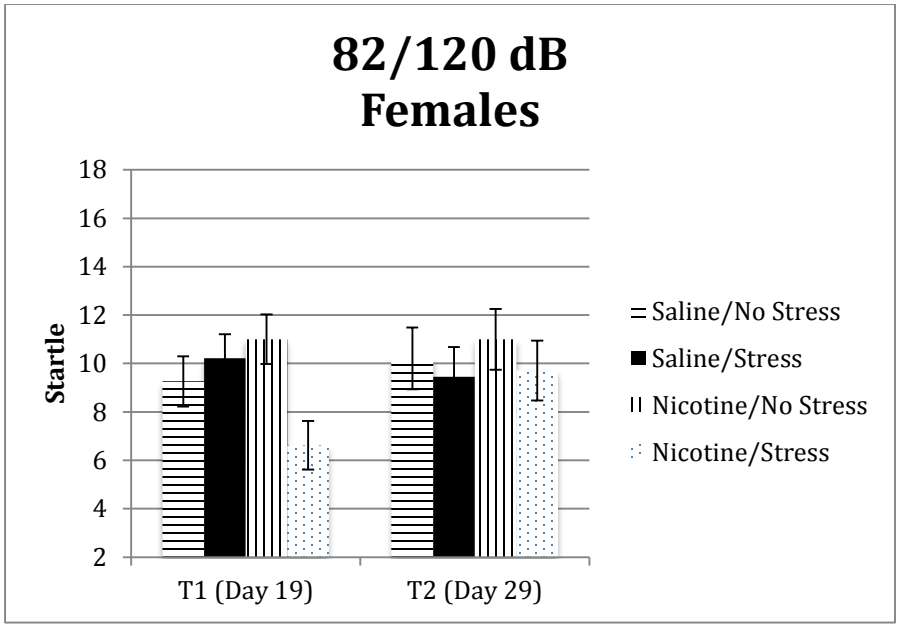
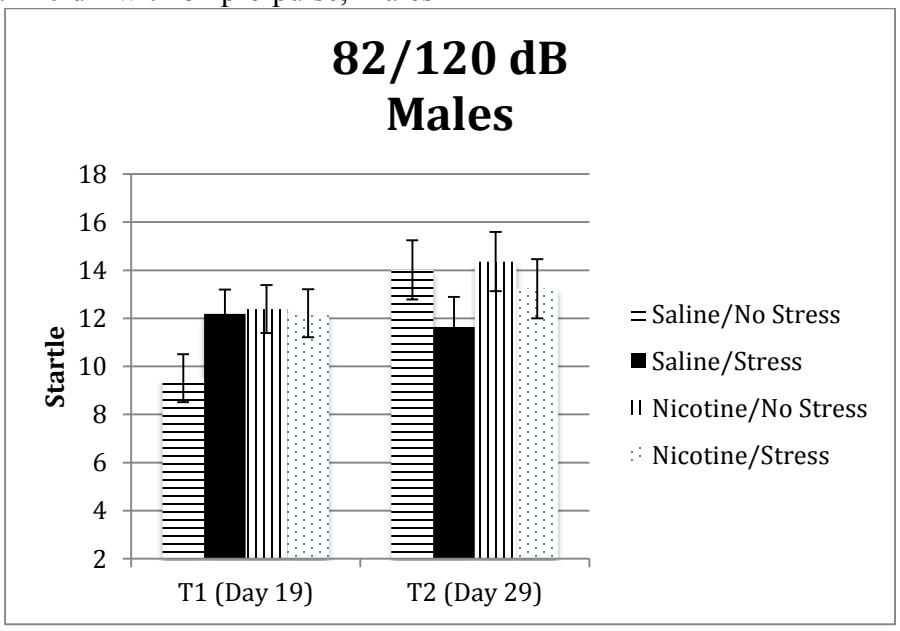


Figure 12. 120 dB with 82 pre-pulse, Males



APPENDIX C: PPI Tables

Table 44. rANCOVA PPI at 110 dB with 68 dB pre-pulse

Within-Subjects, adjusted values

Source	Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Time	204.233	1	204.233	.464	.499	.008	.103
Time * PPI_BL_68.110	315.769	1	315.769	.717	.401	.013	.132
Time * Sex	387.389	1	387.389	.880	.352	.016	.152
Time * Stress	888.307	1	888.307	2.017	.161	.035	.287
Time * Nicotine	64.481	1	64.481	.146	.703	.003	.066
Time * Sex * Stress	438.283	1	438.283	.995	.323	.018	.165
Time * Sex * Nicotine	1329.495	1	1329.495	3.019	.088	.052	.400
Time * Stress * Nicotine	723.078	1	723.078	1.642	.205	.029	.242
Time * Sex * Stress * Nicotine	.009	1	.009	.000	.996	.000	.050
Error(Time)	24222.154	55	440.403				
a. Computed using alpha = .05							

Between-Subjects, adjusted values

Source	Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Intercept	3262.796	1	3262.796	4.806	.033	.080	.577
PPI_BL_68.110	112.742	1	112.742	.166	.685	.003	.069
Sex	2609.329	1	2609.329	3.844	.055	.065	.487
Stress	1846.304	1	1846.304	2.720	.105	.047	.367
Nicotine	134.535	1	134.535	.198	.658	.004	.072
Sex * Stress	128.107	1	128.107	.189	.666	.003	.071
Sex * Nicotine	145.685	1	145.685	.215	.645	.004	.074
Stress * Nicotine	2177.495	1	2177.495	3.208	.079	.055	.421
Sex * Stress * Nicotine	13.770	1	13.770	.020	.887	.000	.052
Error	37337.375	55	678.861				
a. Computed using alpha = .05							

Table 45. rANCOVA PPI at 110 dB with 82 dB pre-pulse

Within-Subjects, adjusted values

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Time	16.407	1	16.407	.043	.837	.001	.055
Time * PPI_BL_68.120	1235.501	1	1235.501	3.215	.078	.055	.422
Time * Sex	.929	1	.929	.002	.961	.000	.050
Time * Stress	142.164	1	142.164	.370	.546	.007	.092
Time * Nicotine	80.827	1	80.827	.210	.648	.004	.074
Time * Sex * Stress	942.559	1	942.559	2.453	.123	.043	.337
Time * Sex * Nicotine	413.097	1	413.097	1.075	.304	.019	.175
Time * Stress * Nicotine	4.167	1	4.167	.011	.917	.000	.051
Time * Sex * Stress * Nicotine	365.100	1	365.100	.950	.334	.017	.160
Error(Time)	21135.105	55	384.275				
a. Computed using alpha = .05							

Between-Subjects, adjusted values

Source	Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Intercept	26.346	1	26.346	.050	.824	.001	.056
PPI_BL_68.120	190.545	1	190.545	.360	.551	.007	.091
Sex	1482.640	1	1482.640	2.805	.100	.049	.377
Stress	111.067	1	111.067	.210	.648	.004	.074
Nicotine	73.178	1	73.178	.138	.711	.003	.065
Sex * Stress	209.739	1	209.739	.397	.531	.007	.095
Sex * Nicotine	7.277	1	7.277	.014	.907	.000	.052
Stress * Nicotine	1936.984	1	1936.984	3.664	.061	.062	.468
Sex * Stress * Nicotine	13.044	1	13.044	.025	.876	.000	.053
Error	29074.985	55	528.636				
a. Computed using alpha = .05							

Table 46. rANCOVA PPI at 120 dB with 68 dB pre-pulse

Within-Subjects, adjusted values

Source	Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Time	16.407	1	16.407	.043	.837	.001	.055
Time * PPI_BL_68.120	1235.501	1	1235.501	3.215	.078	.055	.422
Time * Sex	.929	1	.929	.002	.961	.000	.050
Time * Stress	142.164	1	142.164	.370	.546	.007	.092
Time * Nicotine	80.827	1	80.827	.210	.648	.004	.074
Time * Sex * Stress	942.559	1	942.559	2.453	.123	.043	.337
Time * Sex * Nicotine	413.097	1	413.097	1.075	.304	.019	.175
Time * Stress * Nicotine	4.167	1	4.167	.011	.917	.000	.051
Time * Sex * Stress * Nicotine	365.100	1	365.100	.950	.334	.017	.160
Error(Time)	21135.105	55	384.275				
a. Computed using alpha = .05							

Between-Subjects, adjusted values

Source	Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Intercept	26.346	1	26.346	.050	.824	.001	.056
PPI_BL_68.120	190.545	1	190.545	.360	.551	.007	.091
Sex	1482.640	1	1482.640	2.805	.100	.049	.377
Stress	111.067	1	111.067	.210	.648	.004	.074
Nicotine	73.178	1	73.178	.138	.711	.003	.065
Sex * Stress	209.739	1	209.739	.397	.531	.007	.095
Sex * Nicotine	7.277	1	7.277	.014	.907	.000	.052
Stress * Nicotine	1936.984	1	1936.984	3.664	.061	.062	.468
Sex * Stress * Nicotine	13.044	1	13.044	.025	.876	.000	.053
Error	29074.985	55	528.636				
a. Computed using alpha = .05							

Table 47. rANCOVA PPI at 120 dB with 82 dB pre-pulse

Within-Subjects, adjusted values

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Time	594.471	1	594.471	1.350	.250	.024	.208
Time * PPI_BL_82.120	15.790	1	15.790	.036	.851	.001	.054
Time * Sex	78.219	1	78.219	.178	.675	.003	.070
Time * Stress	295.410	1	295.410	.671	.416	.012	.127
Time * Nicotine	61.727	1	61.727	.140	.710	.003	.066
Time * Sex * Stress	571.437	1	571.437	1.297	.260	.023	.201
Time * Sex * Nicotine	83.683	1	83.683	.190	.665	.003	.071
Time * Stress * Nicotine	128.615	1	128.615	.292	.591	.005	.083
Time * Sex * Stress * Nicotine	2.615	1	2.615	.006	.939	.000	.051
Error(Time)	24225.932	55	440.471				

a. Computed using alpha = .05

Between-Subjects, adjusted values

Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power[a]
Intercept	361.072	1	361.072	.799	.375	.014	.142
PPI_BL_82.120	462.104	1	462.104	1.023	.316	.018	.169
Sex	63.947	1	63.947	.142	.708	.003	.066
Stress	117.048	1	117.048	.259	.613	.005	.079
Nicotine	378.521	1	378.521	.838	.364	.015	.147
Sex * Stress	2837.536	1	2837.536	6.280	.015	.102	.692
Sex * Nicotine	1094.651	1	1094.651	2.423	.125	.042	.334
Stress * Nicotine	1563.921	1	1563.921	3.461	.068	.059	.448
Sex * Stress * Nicotine	249.066	1	249.066	.551	.461	.010	.113
Error	24850.456	55	451.826				

a. Computed using alpha = .05

APPENDIX D: PPI Figures

Figure 13. PPI at 110 dB with 68 dB pre-pulse, Females

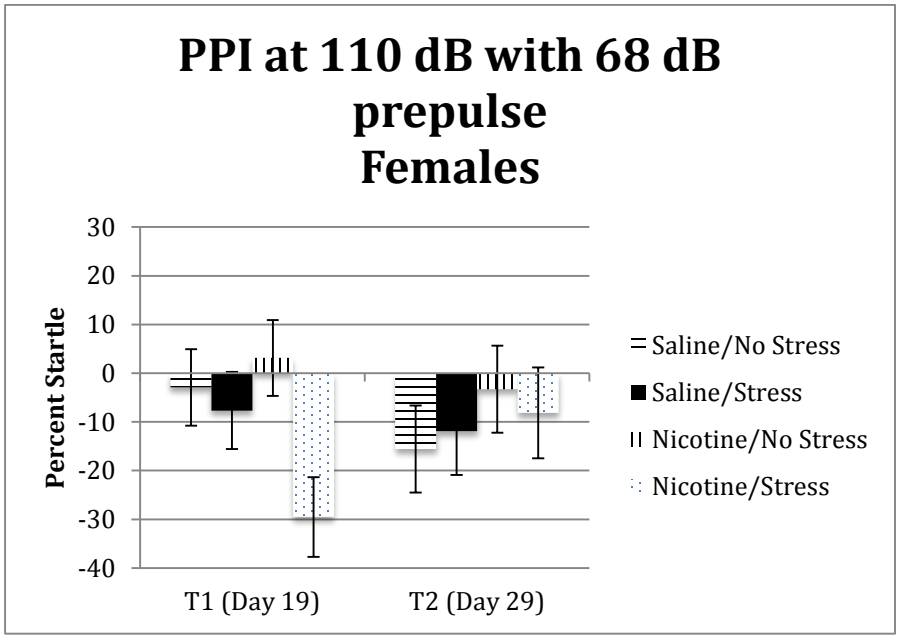


Figure 14. PPI at 110 dB with 68 dB pre-pulse, Males

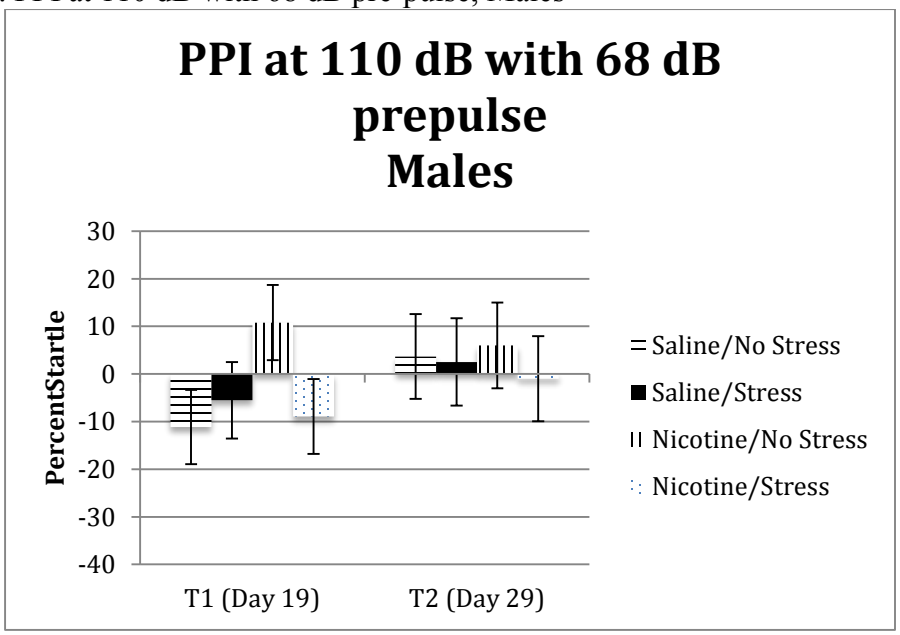


Figure 15. PPI at 110 dB with 82 dB pre-pulse, Females

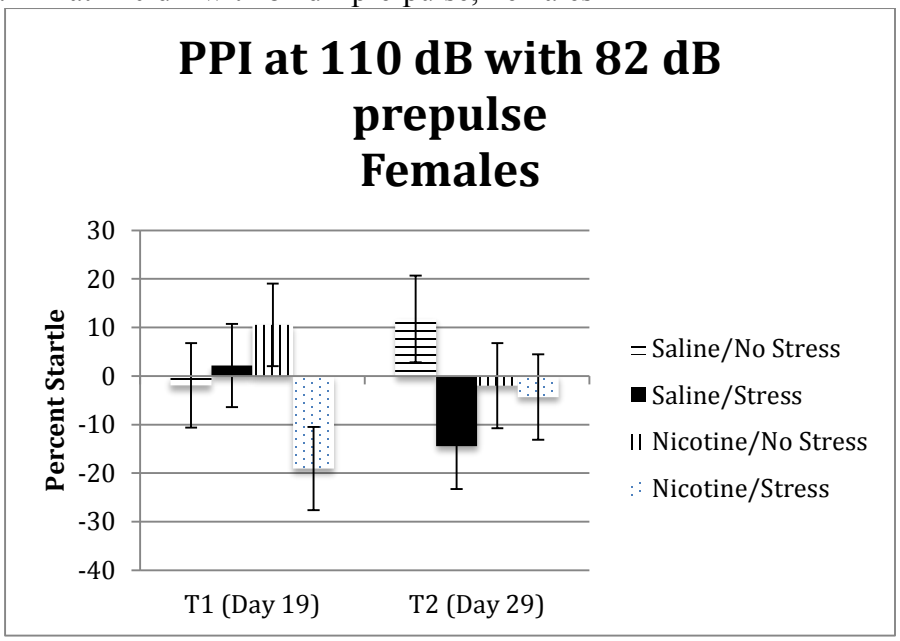


Figure 16. PPI at 110 dB with 82 dB pre-pulse, Males

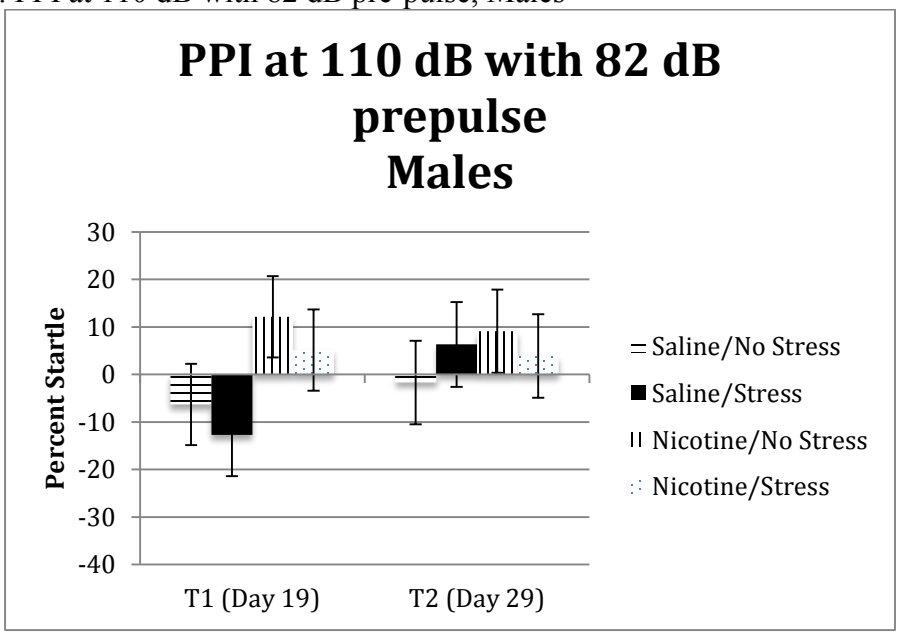


Figure 17. PPI at 120 dB with 68 dB pre-pulse, Females

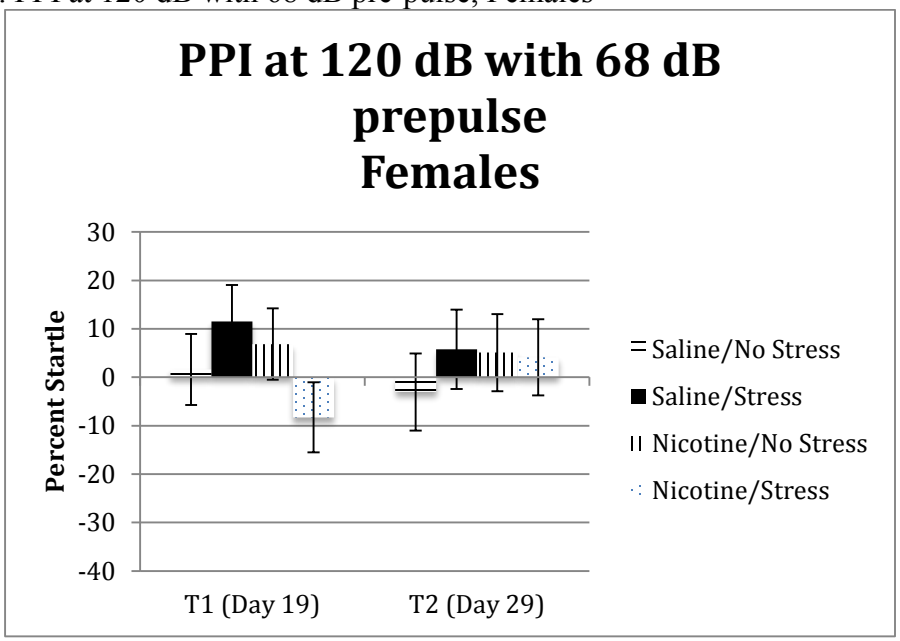


Figure 18. PPI at 120 dB with 68 dB pre-pulse, Males

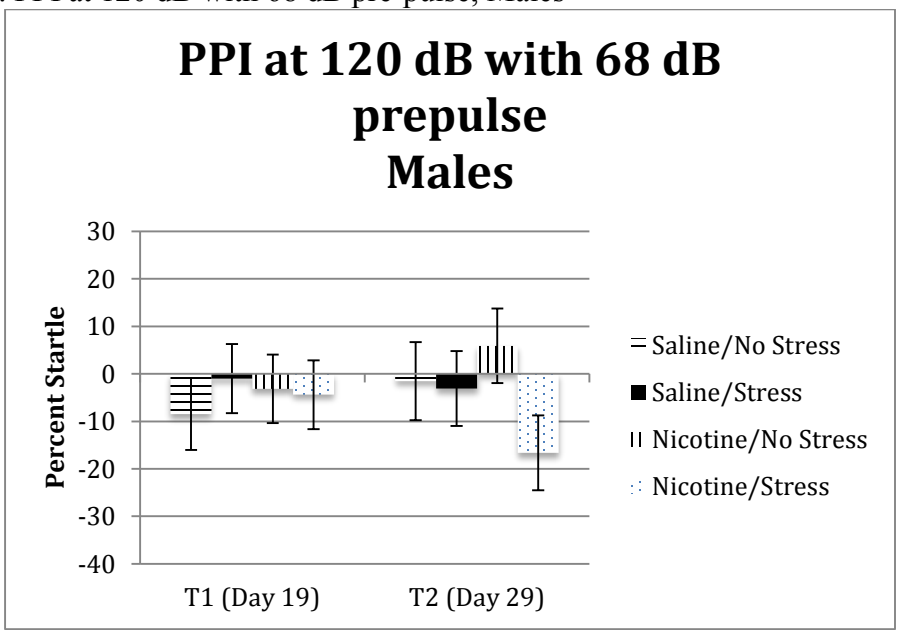


Figure 19. PPI at 120 dB with 82 dB pre-pulse, Females

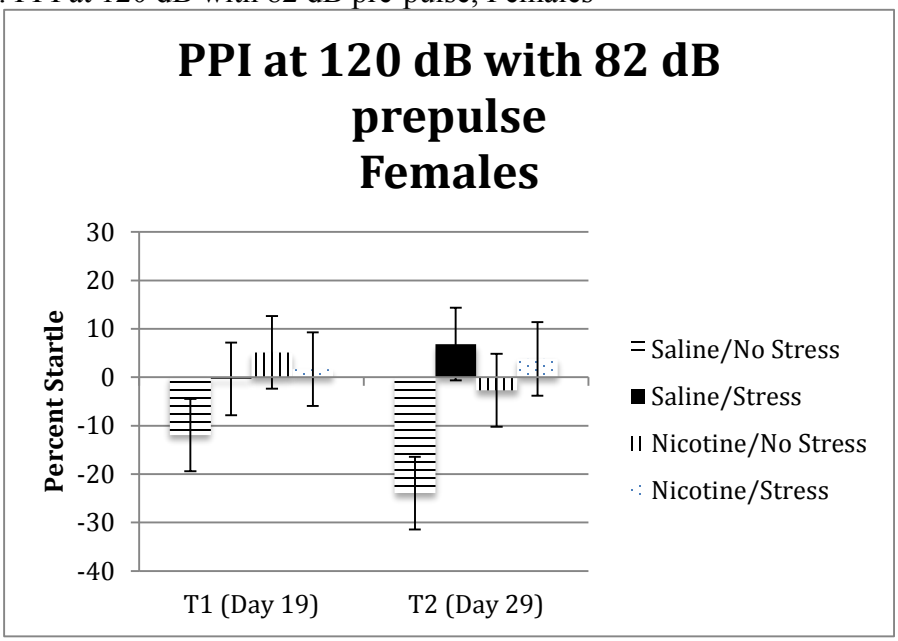
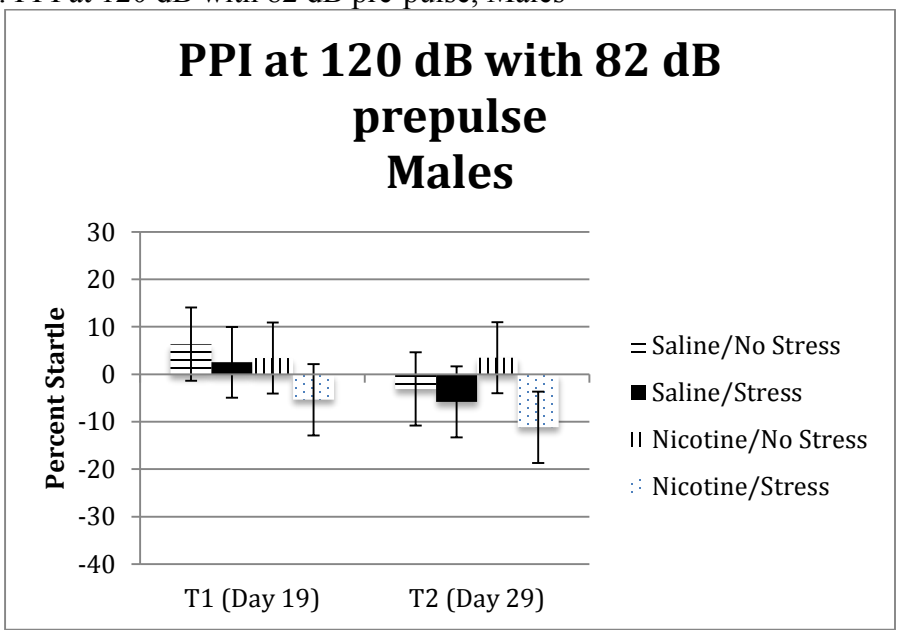


Figure 20. PPI at 120 dB with 82 dB pre-pulse, Males

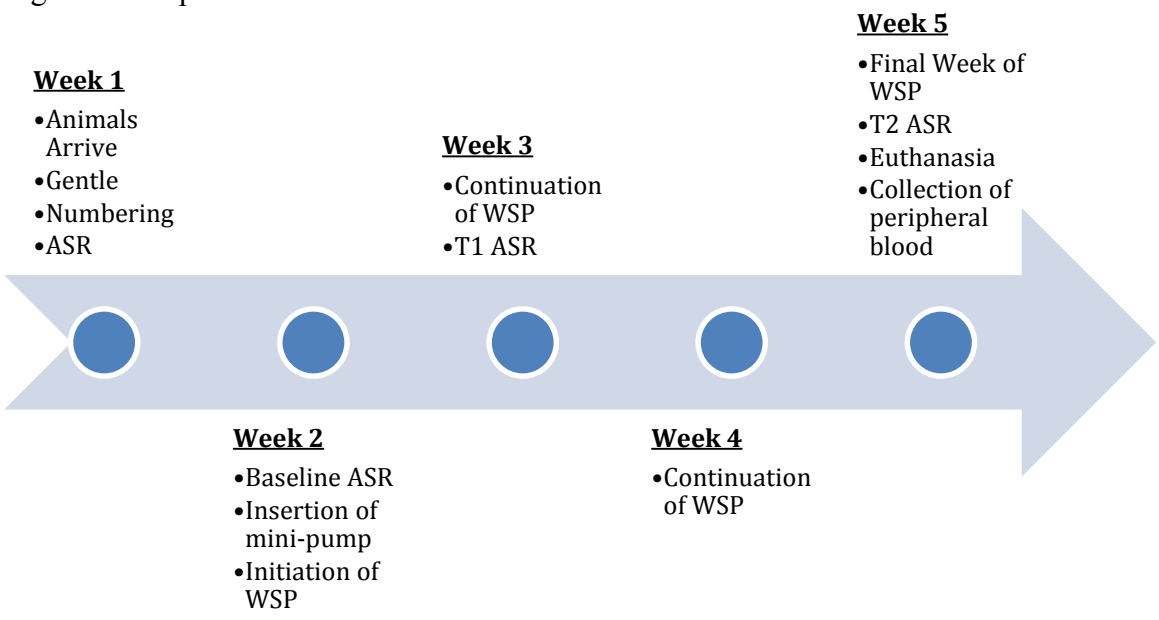


APPENDIX E: Other Figures

Figure 21. Warrior Stress Paradigm Timeline

Timeline of Stress Days		
Stress Day	**Predator Stress**	**Unpredictable Event**
1	**Fox Urine** (20 min)	**None**
2	**Fox Urine** (10 min)	**Whistle** at 12, 15 & 19 min
3	**Fox Urine** (10 min)	**Coin Shake** at 11, 14, & 17 min
4	**Fox Urine** (10 min)	**Flashing Lights** at 13, 16, & 19 min
5	**Fox Urine** (10 min)	**Cage Shake** at 12, 15, & 18 min
6	**Fox Urine** (10 min)	**Flashing Lights** at 12, 16, & 19 min
7	**Fox Urine** (10 min)	**Whistle** at 11, 13, 16 & 18 min
8	**Fox Urine** (10 min)	**Coin Shake** at 12, 16, & 19 min
9	**Fox Urine** (10 min)	**Flashing Lights** at 11, 15, 19 min
10	**Fox Urine** (10 min)	**Cage Shake** at 11, 14, & 17 min
11	**Fox Urine** (10 min)	**Coin Shake** at 13, 16, & 19 min
12	**Fox Urine** (10 min)	**Whistle** at 12, 14, 17 min
13	**Fox Urine** (10 min)	**Flashing Lights** at 11, 14, 18 min
14	**Fox Urine** (10 min)	**Cage Shake** at 12, 15, & 18 min

Figure 22. Experimental Timeline



APPENDIX F: Administrative Documents

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4799

April 2, 2014

MEMORANDUM FOR DR.NEIL GRUNBERG, DEPARTMENT OF MEDICAL AND CLINICAL PSYCHOLOGY

SUBJECT: IACUC Approval of Protocol – Initial Review

The following application was reviewed and approved by the Uniformed Services University of the Health Sciences (USUHS) Institutional Animal Care and Use Committee (IACUC) via Designated Member Review on April 2, 2014:

Title of Application: "Behavioral investigations of nicotine and caffeine in rats (Rattus norvegicus)"

USUHS Protocol Number: MPS-14-898

Expiration Date: April 1, 2017

Supporting Grant(s) Number: E072194414

Name of Principal Investigator: Dr. Neil Grunberg

The USUHS has an Animal Welfare Assurance on file with the Office for Laboratory Animal Welfare (OLAW), National Institutes of Health (NIH). The Assurance Number is A3448-01. The IACUC approved the above referenced application as submitted.

An annual review is required for each of the three years of this protocol. This review must be completed by the anniversary date of the protocol. If work is to be continued past the expiration date, a triennial review must be completed prior to the expiration date in order for work to be uninterrupted. Protocol expiration dates may not be extended, and no animal work may be done without an approved protocol. Although the IACUC may send reminders, it is the investigator's responsibility to submit an annual review form (Form 3206A) at least 30 days in advance, or a new Form 3206 for triennial review at least 60 days in advance of expiration.

Prior to placing your first animal order, please contact MAJ. Amanda Christy to schedule a pre-protocol planning meeting (295-3708). This meeting must occur to ensure animal numbers are loaded in the CART system and LAM resources are available to meet your needs.

Brian M. Cox, Ph.D.
Chair, Institutional Animal
Care and Use Committee

cc: Office of Research

USUHS FORM 3206B
ANIMAL STUDY PROTOCOL
(modification/addendum)

Animal Protocol Number:	MPS-14-898
Grant Number:	E072194414
Principal Investigator:	Neil E. Grunberg, Ph.D.
Department:	MPS
Phone:	295-9673

Animal Protocol Title: Behavioral investigations of nicotine and caffeine in rats (Rattus norvegicus)
(If animal species not identified in title, list parenthetically after title, i.e., rat, mice, etc.)

Grant Title (if different from above): Behavioral effects of stress, nicotine, and caffeine in male and female rats

Description of the proposed modification:

Significant changes to an Animal Study Proposal must be submitted to the USUHS IACUC for review and subsequent coordination for approval. Significant changes include, but are not limited to, a change in one or more of the overall aims or objectives of the study which would affect animal use; an increase in animal pain, distress or discomfort; a change of principal investigator; a change or addition of personnel having a role in the care and handling of animals as approved in the original proposal; a significant change in the animal number; a major change in procedures, surgery, or treatment; addition of the use of hazardous or radioactive materials; a change in species.

Explain the proposed modification(s) to the study. Include a brief description of the reasons for the change(s). Use additional sheets if necessary.

We request the following changes to our protocol:

1. Use of 24 pilot animals to assess preferable route of administration.
2. Change in vehicle of administration for caffeine from drinking water to Jell-O.
3. Addition of the elevated plus maze (EPM) as another behavioral measure of anxiety-related behaviors.
4. Separate housing for male and female animals.

(1, 2) Based on an additional literature review, we would like to pilot drinking water versus Jell-O as potential routes of administration for caffeine. The purpose of this investigation is to enhance experimental control. Based on the lack of precedence of Jell-O administered caffeine, we request the pilot animals to determine appropriate dosage levels, tolerance effects, and confirm whether this method of administration is preferable to drinking water. Two mL of sugar-free Blackcurrant Jell-O will be mixed with the assigned amount of caffeine. The Jell-O will be placed in a small bowl or cup in the rat's cage. These methods are consistent with Leach and colleagues' (2010) study involving Jell-O administration. We would also like to add an acute caffeine administration via oral syringe. Animals are trained to drink out of the syringe. This method was identified as an effective substitute to intrusive methods of involuntary oral dosing (e.g., Schleimer et al., 2005; Atcha et al., 2010). To better model the warrior stress experience, we want to include both chronic caffeine administration as well as acute doses prior to stressors.

To serve these aims, a 2 (female versus male) x 2 (Jell-O versus drinking water) x 2 (control

versus caffeine) factorial design will be used, with 3 animals per cell. Table 1 describes the planned dosing methods that will be used to compare the routes of administration and assess tolerance effects. The pilot animals will be housed for two weeks. Data from the elevated plus maze and open field activity will be collected at multiple time-points to observe behavioral effects of different caffeine administrations. These data will be used to make a final decision on which route of administration to use for the study. Time animals will be tested will not exceed 1 hour/day (only 1 behavior/day).

Table 1: Caffeine dosage plan for pilot animals

Route	Jell-O	Drinking water	Caffeine dosage
Acute dose	20 mg/kg **Jell-O** 30 min before behaviors	20 mg/kg **syringe** (oral) 30 min before behaviors	20 mg/kg in a 2 mL 5% aqueous sucrose solution
Chronic dose	2 x 40 mg/kg Jell-O per day	Ad libitum (1 g/L; avg 100 mg/kg/day)	80-100 mg/kg

(3) We would like to add the elevated plus maze (EPM; Pellow et al., 1985) as an additional behavioral measure. EPM is an established measure of anxiety-related behavior in rodents. The addition of this measure is consistent with the initial study aim to investigate the impact of caffeine on the stress response as demonstrated by various proxies of anxiety in rats. We would like additional measures of anxiety-related behavior to enhance validity of our study. EPM requires that the animals are recorded in the apparatus for five-minutes at each data-point. The EPM apparatus consists of two intersecting wooden bars, which create four arms. Two arms are closed in by walls and the other two arms are open. Anxiety-related behavior in EPM is demonstrated by the longer time spent in the enclosed spaces and less entries into the open arms. After the animals are recorded for five minutes in the apparatus, they are returned to their home cages. For the current study, we plan to collect EPM data across four time-points (two baseline measures & two post-stressor measures). The additional impact on the animals in terms of time and pain is negligible. This measure has been used previously in our lab for over ten years (e.g., Elliott et al., 2004; Elliott et al., 2005).

(4) We would like separate housing due to rats' highly sensitive olfactory senses and the influence opposite-sex pheromones may have on the behavior of both male and female rats (e.g., Zufall & Leinders-Zufall, 2007). We have concerns that this influence may confound our results.

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Principal Investigator Signature — **Date** 7/11/14

Department Chairperson or Activity Head — **Date** 7/11/14

Veterinary Pre-review Signature — **Date** 7/11/14

USUHS Form 3206-B (Revised February 2013)

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